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Now reason can prevail

SIR Gordon Wolstenholme, Chairman of Britain's Genetic Manipulation Advisory Group, said on London Weekend Television last Sunday that GMAG's new system of guidelines for assessing the risks of genetic manipulation experiments "will be so full of sense in the end that it will be the one which will predominate." GMAG has not yet formally adopted the system (which is based on a version of numerical risk analysis) and has opened the subject for debate with the article below (p 104).

The revised guidelines proposed by the US National Institutes of Health are in many ways arbitrary. They are thus open to bitter public attack. The new GMAG system, on the other hand, is rational and therefore more defensible; and it is flexible in that experiments on risk are encouraged and new data can easily be incorporated. GMAG should be encouraged to adopt the new system. But GMAG's path is not strewn with roses.

First, there are internal problems. While the procedure outlined below is clearly rational, it still leaves many of the cards in GMAG's hands. 'Qualitative' biological judgements have to be made alongside the calculations, and it would help if GMAG were to be more explicit about these judgements. It would help also if GMAG's existing case law—which will still be applied where data is not available to support the more scientific system—were to be published.

It would also help if the history of the more numerical parts of the procedure were known. Sydney Brenner had the original idea of calculating a number of separable 'risk factors' for each experiment (last week he said: "I don't believe anything until I see numbers"). His idea was developed in the Safe Vectors Sub-Committee of GMAG, where a trial was run of numerical assessment. Individuals were given a list of experiments whose risks were to be calculated; but they came up with very different figures. However the *ranking* of the experiments with respect to risk was highly consistent, and hence the stress on the use of the figures for rank rather than as absolute numbers in the GMAG document. Also, there is no mention of a 'scale-up' factor in the report. The system is flexible enough that one could be included, but it is not the easy matter it might at first seem. As one GMAG member said "which would you be more afraid of: an experimenter dropping a one-litre glass flask, or an escape from a sealed stainless steel industrial plant containing 10,000 litres?" These and other matters should be aired in the debate to follow, and the new procedure should become better understood and refined in the process.

The second problem GMAG faces is international. GMAG—unlike the National Institutes of Health in the US—controls industrial research as well as academic work. So—certain industrialists argue—British firms will be penalised in the early stages of the potentially enormous business of biotechnology if GMAG's controls are any stiffer than those adopted voluntarily or by fiat elsewhere. It is important for industry that the controls on this research are internationally uniform. But the NIH guidelines, for

example, take no care of what GMAG calls the 'expression factor'—whether the foreign gene is translated into protein or not. As industry clearly wants expression (that gives it its product) it is to industry's advantage that that factor is ignored. And this is just one example. To make such things uniform internationally is no easy matter, and it is important that it should be pursued at the highest government level.

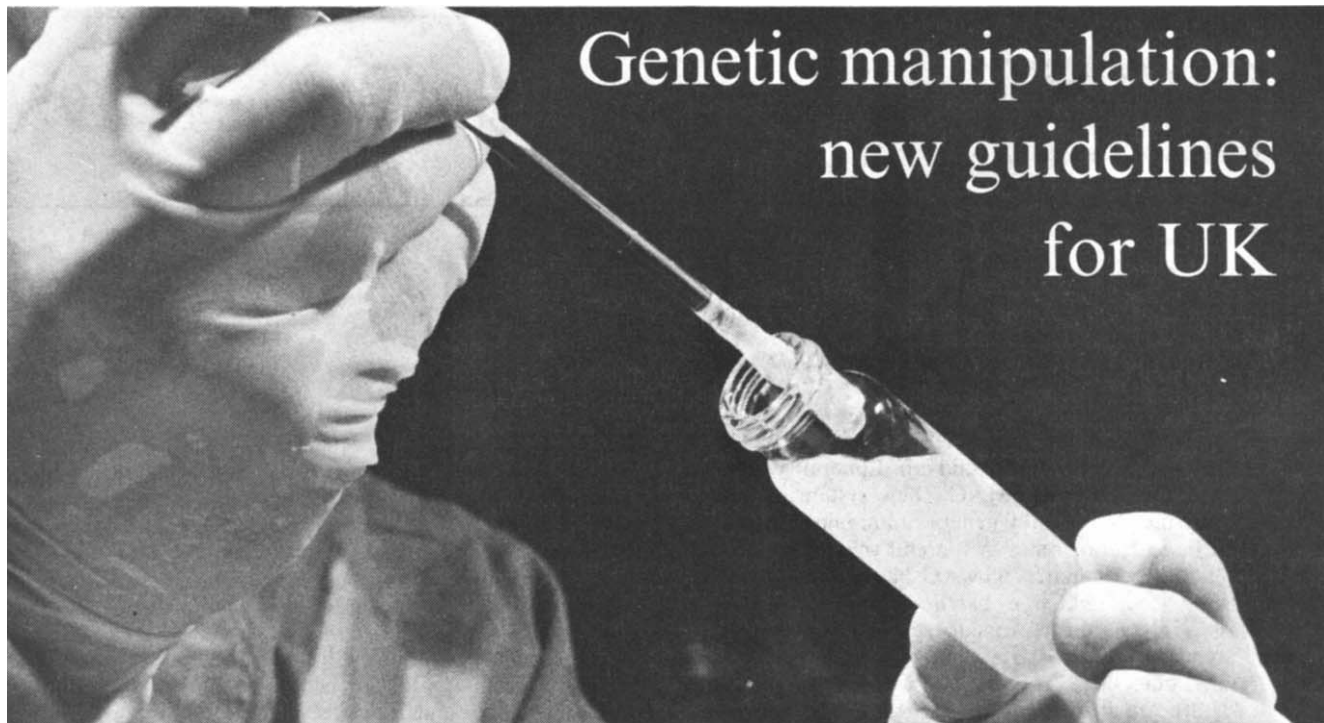
International uniformity becomes a further cause why GMAG must make its procedures crystal clear. The European Science Foundation's Liaison Committee on genetic engineering adopted the earlier British (Williams) guidelines used by GMAG; but its members were lost in applying them to their own countries. What exactly were GMAG's internal procedures? And how could the smaller countries apply them, where there were too few researchers to set up a GMAG equivalent? The NIH system has won in many countries for its transparency—its look-up table of containment—despite its evident logical shortcomings. Europe needs a European GMAG. Perhaps the ESF Liaison Committee itself could take on the role?

The third problem facing GMAG is the research on risks it so welcomingly recommends. How is it to be done? Who is to do it? And who pays? Surprisingly, perhaps, the last question is the easiest to answer. Britain's Minister of State for Education and Science, Mrs Shirley Williams, interviewed last week on London Weekend television by MP Brian Walden, promised "tens of millions of pounds" for risk research in Britain. With that much, risk research might become like cancer research in the US: everybody will be doing it because the money is there. £10 million would certainly answer the previous question—who is to do it?

There remains the problem of careers for 'riskers'; as Brian Walden put it "there are no Nobel prizes for risk research". (Though who knows? One of the risks is of the escape of a bacterium producing large amounts of insulin, and no-one knows what such a bug would do. Another risk is of a bacterium expressing, say, cell surface proteins upsetting the immune system and inducing an autoimmune disease. Again, too little is known about the immune system to predict the consequences. Experiments on such matters might throw up new knowledge.) But if university researchers cannot be drawn away from their present interests what of the civil service scientists? What about those ex-Ministry of Defence people at Porton Down, now under Shirley Williams' control? Could they begin work on risk research?

Then there is also the problem of how risk research is to be commissioned. Surely GMAG should do that. GMAG has already expressed a willingness to do so, through the 'technical panel' GMAG recommends in its new proposals. And one final point. That panel should not consist entirely of academics, as its name appears to suggest. One of GMAG's strengths has been the breadth of interest represented by its members. It should not abandon that strength in what will become its inner sanctum. □





Genetic manipulation: new guidelines for UK

● Britain's Genetic Manipulation Advisory Group (GMAG) is preparing to adopt a new approach to assessing the risks of 'genetic engineering' experiments. GMAG's statement—approved in principle at a meeting on 2 November—appears below. The scheme is based on an attempt to assign numerical risks to individual experiments as a product of three factors: the 'access factor', the 'expression factor', and the 'damage factor'. But the numbers are used only to rank the risks so that the experiments can be assigned consistently to the containment categories I to IV. Qualitative matters are also taken into account, for biology has not yet been reduced entirely to numbers.

The scheme will be used when there are enough data to employ it; otherwise assessment will revert to the old Williams 'phylogenetic' guidelines. Its great virtue appears to lie in its objective scientific basis and its consequent encouragement to research on risks.

GMAG's Sub-Committee on the Validation of Safe Vectors* and, more recently, an *ad hoc* Working Party on Guideline Criteria†, have been considering on GMAG's behalf the merits of an alternative approach to the categorisation of genetic manipulation experiments first outlined by Dr Sydney Brenner (MRC Laboratory of Molecular Biology, Cambridge). GMAG, having considered the enthusiastic advice it has received, is persuaded of the general soundness of the new system, and subject to the consideration of comment on this dis-

cussion document and consultation with the Department of Education and Science and the Health and Safety Executive, hopes to introduce the new principles—to be used for the time being in parallel with the existing Williams Guidelines'—early in 1979. Before refining the system, GMAG wishes to take into account the opinions of the scientific community, and it is therefore hoped that publication of the following will allow all concerned to make their views known to the Group‡. In the very near future, GMAG will be arranging a meeting with users and with members of local Biological Safety Committees.

While seeing justification and opportunities for change, GMAG considers that there is no case for changing the general arrangements governing this area of research in the UK: the definition of genetic manipulation remains unchanged; the regulations² to notify work being done must apply; the roles of GMAG and HSE have been established; facilities for physical containment (categories I-IV) have been defined and substantial investment has gone into their construction.³ Proposals are put forward in this paper, however, which if implemented would lead to change in the scientific considerations upon which the categorisations of individual experiments are decided by GMAG.

At present, GMAG broadly follows the Report of the Williams Working Party in allotting experimental protocols to the four categories of physical containment. The chief conclusion of the arguments which follow is that those experiments for which it is possible to carry out an approximate

risk assessment should where appropriate be given categorisations distinguished by the labels I*, II*, III* and IV*. (It must be emphasised that no change is implied in the specification of physical containment facilities, but only in the categorisation of certain experiments.) It is suggested that the two systems of categorisation should persist in parallel for as long as may be necessary. At this stage relatively few experiments can be given the new starred categorisations, but a steady transfer of experiments and classes of experiments from one system to the other should be possible.

The Williams guidelines

Although in the past two years GMAG has based its advice on the "suggested categorisations" of the Williams Report, there have been several occasions when it has been necessary to modify or to extend that scheme. That this should have been so is not surprising, for the Williams Working Party reported when there had been very little practical experience of genetic manipulation. Moreover, the report explicitly acknowledged that its categorisations were likely to be found insufficient in some respects, and it

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foresaw the need for refinement and substantial modification.

First, doubts have accumulated about the value of the criterion of phylogenetic relatedness as a measure of the possibility that genetic manipulations may be hazardous. A simple illustration will show the difficulty. According to the suggested categorisations, genetic elements from plants entail less relative hazard (to human beings) than genetic elements from higher organisms, yet it is also clear that the incorporation into the genome of a strain of *E. coli* viable in the human gut of a gene responsible for a plant immunogen (say the glycoprotein gluten from wheat germ) could be relatively more hazardous than a gene responsible for the production of, say, human globin. More generally, the bearing of phylogenetic relatedness on the potential dangers of experiments in genetic manipulation must be modified by the nature of the host and the vector; animal genes are, for example, less likely to retain their original functions when incorporated in the genomes of bacteria than in those of animal cells or viruses.

Second, the experience of the past two years has also shown that there is a need for a modification of the Williams prescription about the identity and the purity of transplanted genetic elements. Often the more relevant characteristic is whether the foreign gene is capable of being expressed (or otherwise retaining its original function). Moreover, evidence has accumulated in the past two years to suggest that many of the genes of higher organisms are distributed as separate pieces among the genetic material of the donor organism, which decreases the likelihood that shotgun experiments in which pieces of random DNA are transferred to other (and especially bacterial) hosts will yield functional genes in the new genetic environment. On the other hand, experiments in which genetic elements are obtained in potentially functioning form from functional molecules of messenger RNA, often regarded in the past two years as an assurance of their purity, may be in principle more potentially hazardous than the same genetic elements obtained directly from the genomic donor DNA. These statements are not, of course, of general application, but they are another reason to believe that individual cases should be dealt with separately.

Considerations of the host-vector system remain generally applicable. If there are hazards in any experiment in genetic manipulation, it will plainly be beneficial that a host organism carrying foreign genetic elements should be incapable of infecting human beings, and that the vectors used in the experi-

ments should be specific to these host organisms. Appropriate use of "disabled" host-vector systems, offering "biological containment", will continue to be one of the essential features of certain types of recombinant DNA research.

More objective methods

In the circumstances, it is likely that all interested parties, scientists and the general public alike, would benefit if there were a more objective and even quantitative basis for assessing the risks of genetic manipulation. But it will be some time, perhaps several years, before biological understanding and quantitative data have accumulated sufficiently for such techniques to be applied to all proposals for experiments in genetic manipulation.

Elements of risk assessment

Assessment of the risk of experiments in genetic manipulation must follow a formal and comprehensive risk analysis, which is possible only when the biology of the host-vector system and of its interaction with the cells of organisms at risk is reasonably well known. At present the relevant biological data are not generally available, and many systems of potential value in genetic manipulation will therefore be excluded from the categorisation procedure now proposed. With the passage of time, however, it will be possible to include within the new basis for categorisation an increasing proportion of experimental proposals.

In general, risk analysis entails a consideration of the following steps.

(a) *Escape from containment*

The escape of organisms carrying foreign genetic elements from the containment specified for them is always a possibility, either because the facilities are not designed in such a way as to provide absolute assurance that organisms will not escape or because of some malfunction or accident. Thus it is proper to associate with each category of containment a specific probability (C_i , where i runs from I to IV) that organisms will escape. The quantities C_i have the units "probability per unit time per organism contained".

The ratios of these probabilities will be measures of the relative efficacy of the four different levels of containment, and there are at present only rough estimates of the ratio C_{IV}/C_I , the relative safety of the most stringent and the least stringent of the containment categories now in use. The ratio is likely to be less than 10^{-6} (corresponding to a factor of 100 for the interval between each category of containment) and may be less than 10^{-9} . It is important that the true range of safety offered by the four categories

of containment should urgently be defined more precisely. On present indications, the range of efficacy spanning the least and the most stringent categories of containment is comparable with the degree to which the viability of some host organisms is reduced relative to the wild-type strains; this points to questions for the future about the relative protection from conjectured hazard of physical and biological containment.

(b) *Persistence in the environment*

The most direct concern in the assessment of the risks of genetic manipulation is that organisms carrying foreign genetic elements will gain access to the tissues of laboratory workers, but it is important that those concerned with the safety of genetic manipulation should consider the possibility that, as an intermediate step, organisms may lodge and even replicate in the laboratory into which they have escaped (as, for example, in culture dishes). In this and other parts of the analysis, care must be taken to identify all the possible circumstances in which organisms carrying particular foreign genetic elements may be relatively at an advantage, so that the genes concerned are amplified and not attenuated.

(c) *Access to organisms at risk*

Organisms which escape as aerosols may be inhaled by laboratory workers or ingested, thus gaining access directly to the nasal tracts, the lungs and the gut. It is important that unfamiliar routes of access should not be overlooked—for example, the effects on human lungs of the inhalation of *E. coli* organisms carrying genes capable of producing, say, insulin are at present unknown.

(d) *Replication*

The risk analysis of experiments in genetic manipulation differs from that of, say, the operation of nuclear power plants in that agents which may potentially cause damage may in some circumstances be capable of replication. In the risk analysis of experiments in genetic manipulation, great care should therefore be taken to identify pathways that permit the replication of organisms carrying foreign genetic elements or which permit the transfer of the foreign genetic elements to other organisms which are capable of replication. One factor in a quantitative measure of a particular hazard would no doubt be provided by the duration and the intensity of the exposure of susceptible cells to organisms carrying potentially damaging genetic elements, and it is theoretically possible to calculate quantities such as these, given assumptions about the viability of manipulated organisms in the environments in which they lodge. Such formal calculations are however unlikely to be

Examples of the new categories

● The cloning of pure mammalian globin genes in an approved disabled *E. coli* host-vector system in circumstances in which the globin genes are not expressed would be I*. For the time being, the use of non-disabled strains of *E. coli* (including K12) or the expression of the globin genes in a disabled host-vector system would place the experiment in category II*. The cloning of expressible globin genes in a non-disabled host-vector system would, for the time being, be a category III* experiment. The allocations to these higher categories are justified by the present lack of certainty that the secretion of mammalian globin in the body tracts (including lungs and the genito-urinary tract) to which *E. coli* has access would be without risk. As this uncertainty is removed, lower categorisation may be considered appropriate.

Category I* is justified for the cloning of non-expressible

globin genes in a disabled host-vector system on the following grounds—all three of the factors *A*, *E* and *D* are small (notionally less than unity). If it emerges that *D* is small even when globin genes are expressed, the experiments provisionally allocated to II* and III* might be reduced in categorisation.

This experiment may therefore be considered typical of those destined for Category I*. It should be noted that two of the three factors afford a measure of biological protection, and that in spite of the apparent innocuousness of globin, GMAG would probably be unwilling to agree that an experiment of this kind should be undertaken with wild-type *E. coli*.

● The cloning of genetic elements from the genome of the mouse with the use of defective polyoma virus as a vector, and with cultured mouse cells as hosts (with appropriate helper virus to facilitate the replication of the virus and the expression of functionable genes) would also

meaningful in present circumstances, so that more qualitative comparisons must at present suffice.

Great care must be taken to ensure that full allowance is made for the unavoidable incompleteness of present knowledge of the behaviour of foreign genetic elements in their hosts. Thus allowance must be made for the possibility that a "disabled" strain will revert (by genetic mutation) to the wild type, or that a foreign gene will be transferred (by the mobilisation of the plasmid) to wild-type bacteria. As things are, sufficient numerical data do not exist bearing on the chance that such events will occur, so that, for some time to come, researchers will have to rely on order-of-magnitude estimates of some of these quantities.

The conjectured hazards

Concern that genetic manipulation may entail hazards for living things stems from the possibility that foreign genetic elements may affect the normal functioning of particular groups of cells or the coordinated functioning of organisms at risk. Another of the impediments to formal and quantitative risk assessment that is likely to persist for some time is that of comparing the possible (but still hypothetical) biological consequences of foreign genetic elements with the damage done by naturally occurring disease. Even so, a tentative listing of some of the possible interactions between foreign genetic elements is suggestive of some of the points to which researchers should direct attention.

(i) Cell death

Cells may be killed when they are infected by viruses or when they are exposed to toxins produced by bacteria. Specific microorganisms and viruses often affect specific groups of cells in the body of a higher organism—polio-myelitis kills people because the polio-myelitis virus kills cells in the central

nervous system when it has access to them, for example. In the analysis of the risks of genetic manipulation, the questions arise whether bacteria or viruses carrying foreign genetic elements are more virulent than the originals, whether the range of their specificity has been extended and whether bacteria carrying the intact genomes of pathogenic viruses make it possible for cells at risk to be exposed to potentially damaging viruses by unnatural routes.

(ii) Changes of genetic constitution

The introduction of foreign genetic elements into human somatic cells could have a number of harmful consequences. The state of differentiation of the cell might be affected. Cryptic viral elements already included in the DNA of the cell might be mobilised, or the foreign genetic elements themselves might be incorporated in the genome of the intact cell, increasing the range of cryptic viral function embodied in the cell. The chief causes of anxiety on such grounds have so far been in connexion with the occurrence of cancer, but understanding is far from complete. Experiments in which animal viruses multiply in human cells (in tissue cultures) without killing the cells may deserve special attention.

(iii) Immunological consequences

There are several hypothetical mechanisms whereby untoward immunological reactions might be provoked by foreign genetic elements. Thus foreign genetic elements might be incorporated in the genome of a human cell in such a way that the product of that gene (possibly a protein) was expressed on the cell surface, thus exposing the cells affected to attack by the body's immunological defences; this may correspond to the mechanism of some naturally occurring autoimmune diseases. On the other hand, the introduction of foreign genetic elements might be accomplished in such a way that their presence



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simulates that of the normal components of body tissues, with the result that the range of the sensitivity of the immune system would be narrowed. In principle, it is also possible that some of the cells of the immune system are especially vulnerable to organisms carrying particular genetic elements.

(iv) *Disturbances of endocrine function* Many hormones are believed to exert their physiological effects by interacting with specific biochemical elements either on cell surfaces or in the nuclear DNA. Foreign genetic elements could in principle interfere with either of these reactions, but it is also the case that infection by bacteria carrying genes which direct the synthesis of human hormones and which are expressed and then secreted into the body could be damaging to the individuals concerned.

(v) Disturbances of cell metabolism

Foreign genetic elements inadvertently introduced into somatic cells could in principle disturb the metabolism of these cells, changing their metabolic rate, the rate of cell division or even the process (still unknown) by which somatic cells age.

(vi) *Consequences for microorganisms* Experiments in genetic manipulation in which bacteria might artificially be en-

be a category I* experiment. Formally, such experiments have much in common with those in which fragments of the *E. coli* genome are redistributed within the same organism with the help of *lambda* bacteriophage. In reality, however, the possibility that polyoma virus may replicate in cells other than those of the mouse cannot be entirely discounted (replication in rat cells has been demonstrated, while the possibility that polyoma might replicate in human cells has not been vigorously explored). Accordingly, the cloning of pure mouse genes in polyoma and cultured mouse cells is suggested as a category I* experiment, with mouse-genome shotgun experiments in category II* for the time being.

● Category I* will also include those experiments for which at present there is no substantial evidence of risk. The chief candidates of this kind are the experiments in which *E. coli* genes are cloned in *E. coli* organisms.

● If these illustrations are taken as a paradigm of

experiments in category I*, then it would follow that disabled *E. coli* carrying but capable of expressing the gene for, say, wheat germ gluten would be categorised II* to allow for the possibility that at least some members of the population might react immunologically against the production of gluten in such a way. Other experiments that might be given the same categorisation on further consideration by the technical panel might include the incorporation of non-expressed interferon genes in *E. coli* K12, or expressed interferon genes in a more disabled strain of *E. coli* K12 (eg χ 1776). Shotgun experiments with mouse DNA cloned in polyoma virus would also be included.

● Similarly, and again on further examination by the technical panel, category III* might include disabled *E. coli* strains carrying expressed human growth hormone genes; the expression of cholera toxin genes in disabled strains of *E. coli* would remain in category IV*.



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dowed with resistance to antibiotics of medical or veterinary importance are potentially hazardous. Another early suggestion of a damage mechanism is the possibility that apparently innocuous organisms (such as the laboratory strains of *E. coli* K12) could be converted by genetic manipulation into infectious pathogens; relevant in this regard is the conclusion of a conference held at Falmouth (New Hampshire, USA) in 1977 that *E. coli* K12 at least cannot be converted to an epidemic pathogen. There are three kinds of reasons for this conclusion: the genetic basis of pathogenicity is complex and does not reside in a single gene, the wild-type strains of *E. coli* which have evolved are probably in some sense optimal for the ecological niches they occupy and the viability of laboratory strains of *E. coli* is so much less than that of wild-type bacteria that such organisms carrying pathogenic elements would probably be eliminated rapidly. This conclusion does not imply that individuals could not be damaged by *E. coli* carrying pathogenic elements such as the gene for cholera toxin, but merely that the production of an infectious pathogenic agent is improbable. There is a need for a consideration along the same lines of other

organisms capable of infecting human beings.

(vii) Ecological consequences

Similar considerations apply to the interaction of foreign genetic elements and species other than human beings. To the extent that species were differently affected and that foreign genetic elements were widespread, ecological changes are in principle possible. Changes in the ecological balance of microorganisms are perhaps especially important.

For those assessing recombinant DNA experiments, this list should make it possible to single out those kinds of interactions between foreign genetic elements and cells which are likely to be widely regarded as potentially serious and damaging. Possible immunological and oncogenic consequences point to the need for a relatively high category of physical containment.

Application in the immediate future

In an ideal world, it would be possible to make numerical estimates of the risk of experiments in genetic manipulation by multiplying together the probabilities representing the various events (a) to (d) (see page 105), summing over all possible pathways leading from the escape of a genetically altered organism from its containment to the causation of a particular consequence for cells exposed and then multiplying by factors which take account of the costs of the damage that results. This is not now possible, however. Some may even argue that it will never be possible or necessary.

Nevertheless it is possible to use the framework of this formal risk assessment to rank the relative risks of some experiments in genetic manipulation, albeit in a somewhat qualitative manner. To that extent, the arguments first put forward by Dr Sydney Brenner in the GMAG Safe Vectors Sub-

Committee are a valuable means of moving towards a more objective system of categorisation.

What is now proposed is that experiments with which researchers are familiar should be allocated to the four containment categories on the basis of a qualitative and relative assessment of their risks; and that the potential risks of novel experiments should then be assessed in relation to these familiar paradigm experiments.

For this purpose, it is convenient (as Brenner has argued) to define a group of three factors which may be taken to represent the chance that events following the escape of genetically altered organisms into the environment may lead to particular conjectured hazards. If data were available, it would then be possible to think of calculating the risk of an experiment by multiplying the numerical values of the three factors together. For the time being, however, such a procedure would provide a spurious sense of precision and it is proposed that there should be a comparison (which necessarily entails the exercise of people's judgment) of the values of the three factors relative to those which obtain for the paradigm experiments.

Analysis might therefore proceed as follows. First, it is supposed that there is an escape of organisms carrying foreign genetic elements. The overriding element in the assessment of the risk of a particular kind of disturbance is the likelihood that the organisms or the genetic elements they carry will have access to susceptible cells of the target organisms. Thus there is defined an

● **Access factor (A).** This is plainly a composite of factors representing the chance that escaped organisms will actually enter the human body, that they will survive there and that they will penetrate whatever membranes must be penetrated so as to reach the

tissues containing susceptible cells. For many experiments, biological containment is dominant—for example the disabled strain of *E. coli* K12 known as χ 1776 may be less viable than wild-type strains of *E. coli* by a factor of 10^6 .

In some experiments in genetic manipulation, the potential risk depends crucially on whether the foreign genetic element is able to express its normal function. This would, for example, be the case in an experiment in which the gene specifying the cholera toxin was incorporated in a plasmid of *E. coli*. Thus it is convenient to define an

● **Expression factor (E)**, which would be large (or notionally have the numerical value 1) if the foreign gene were efficiently translated into the protein product of the gene and if the products were then secreted from the altered organism, which would have a similar value in those circumstances (as for example in the carriage of a genetic element which can be integrated into the genome of target cells without being translated) but which would be small in those experiments in which steps had been taken deliberately to ensure that the gene would not be expressed.

Shotgun experiments require further consideration. The fear that "unknown" genetic elements might cause unknown and damaging changes in organisms at risk has been widespread for the past four years. The Williams Report suggested that experiments involving random lengths of DNA should be categorised somewhere in between the categorisation appropriate for genetic elements known to be damaging (eg cholera toxin) and those thought to be without risk. This conclusion remains valid, but knowledge of the genetic constitution of organisms is growing quickly, and for some it is now reasonably certain that a genetic element drawn at random is likely to be less of a relative hazard than one deliberately selected. This, for example, may be the case with many animal viruses, whose genetic structures appear to be so economical of DNA that it is hard to believe that a piece drawn at random would have physiological significance. Thus it is convenient to define a

● **Damage factor (D)**, which expresses the chance that a genetic element will cause damage. *D* will be large (notionally, unity) if, for example, the host organism were known to contain a gene specifying a bacterial toxin; will be less if it is a genetic element drawn at random from the same bacterial genome; and will be very small if the product of the gene is known to be without physiological effect on the cells of the target organism (as may be the

case where the gene product is, for example, human globin).

It is proposed that, in the months ahead, GMAG should take steps to allow researchers (if they wish) to submit their proposals to GMAG with evidence which will allow GMAG to make an assessment of the three factors and advise on the new basis. Given the present incompleteness of biological understanding and the lack of data bearing on the numerical determination of these factors, it is clear that, for the time being, decisions will continue to depend on judgment rather than calculation. It is also clear that experiments using familiar systems will be the most susceptible to this alternative treatment. One advantage of following such a course of action will nevertheless be to encourage the accumulation of data bearing on the ultimately more objective assessment of risks.

Proposals

The particular recommendations that follow are designed so as to achieve the following objectives. First, the intellectual framework of risk analysis should be used as a means of further refining the self-consistency of GMAG's present procedures. Second, GMAG's procedures should remain flexible, and be capable quickly of being adjusted in the light of new knowledge. Third, new criteria for deciding on categorisations should be applied at the outset only to the systems with which researchers are most familiar, with the expectation that, with the passage of time, increasing numbers of experiments will be included.

It is therefore proposed that GMAG should take the following steps as soon as this may be convenient:

1. The present Williams categorisations will be retained, and GMAG's present methods of assessment will be retained for experiments whose relative hazards cannot be assessed in the manner outlined above. But researchers will also be invited to ask that experiments should be given a different categorisation than suggested by Williams if they have reason to believe that in their case the factors *A*, *E* and *D* would justify such a course. Similarly, GMAG should be able to require more stringent categorisation in appropriate circumstances.
2. GMAG will set up a technical panel to scrutinise such applications on the alternative basis and to report to GMAG, to collect data bearing on a more thorough understanding and evaluation of the factors *A*, *E* and *D*, and to publish (through GMAG) to the scientific community advice on the continuing refinement of the procedure. Researchers might have to accept that, initially at least, these complementary procedures might entail some delay.

In the first instance, it would be for applicants to provide such empirical data as they can on which the technical panel's consideration of the three factors would be based. It is hoped, however, that the technical panel would be a powerful stimulus of the research needed to make risk assessment more objective and widely applicable. Refinement of present knowledge of the relative efficacy of the different categories of containment may be thought an urgent need.

3. The proposed system must be grounded by the allocation of some paradigm experiments to the four categories of containment.

4. It is proposed that for experiments of the kind referred to in the box (item (c)), and for others in which the genes of *Bacillus subtilis* and *Pseudomonas aeruginosa* are rearranged, it should be open to laboratories to provide a block notification of a programme of experiments to be carried out over a substantial period of time, say a year. A condition of the acceptance of such a notification will be the inspection of the laboratory by GMAG, an undertaking to work in category I* conditions and to submit a report at some required interval (not less than a year) providing a detailed log of all experiments carried out. Laboratories working to such a block notification would be required to inform GMAG of all new workers in the laboratory, and of the transfer of organisms to other laboratories; but the use of such organisms in recipient laboratories could be carried out under conditions of good microbiological practice (PHLS Handbook No. 4). In due course, this category of experiments without discernible risk will no doubt be extended.

Acknowledgments

The credit for urging on GMAG the risk assessment approach to genetic manipulation and for drawing it first to the attention of GMAG's Safe Vectors Sub-Committee goes to Dr Sydney Brenner, whose cooperation in developing the system into its present form is also warmly acknowledged. GMAG also wishes to thank the Guideline Criteria Working Party for their work in drafting the present paper, and innumerable other experts who have generously given their advice. □

References

- 1 Report of the Working Party on the Practice of Genetics Manipulation. HMSO, Cmd 6600, 1976.
 - 2 Health and Safety (Genetic Manipulation) Regulations 1978. SI 1978, No. 752.
 - 3 First Report of the Genetic Manipulation Advisory Group. HMSO, Cmd 7215, 1978.
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Top Scottish astronomer resigns after staffing row

PROFESSOR Vincent Reddish (above), director of the Royal Observatory, Edinburgh (ROE), handed in his resignation last week to the UK Science Research Council (SRC) which runs the observatory. When he relinquishes the directorship next September, he will also leave two associated posts, Astronomer Royal for Scotland and Regius Professor of Astronomy at Edinburgh University.

Professor Reddish's decision to resign has come after a prolonged dispute with the SRC over filling some senior posts, in particular that of head of technology. Administrators within the SRC would like to see the post go to an engineer. Reddish would like it to be given to an astronomer with some technological experience, who could interchange with astronomers at the observatory's other telescopes in Hawaii and Australia. The resulting deadlock has led to some posts remaining unfilled for years, according to Professor Reddish, and the lack of some senior staff is now beginning to impair the work of the observatory.

The SRC is trying to persuade Reddish to postpone or reconsider his resignation. It would like to readvertise the head of technology post inviting both engineers and astronomers to apply. The ideal candidate, according to an SRC spokesman, would be someone who is primarily an astronomer with a great interest in or some experience of associated engineering. Reddish feels that the reforms needed to make him change his mind are primarily too great for the SRC to implement. "In principle," he says, "the problem is essentially who decides how to do the work here and the kinds of skill needed to do it." In the event of no successful settlement, he plans to retire from astronomy altogether and move into business. □

Appleton and Rutherford to merge

At a meeting on 18 October, the Council of the UK Science Research Council (SRC) took the decision to close down its Appleton Laboratory at Slough and move it to the Rutherford Laboratory, also run by the SRC, at Chilton in Oxfordshire. The reasons for the merger are scientific, says the SRC. "Bringing these teams together would be the best way of doing their scientific work", according to Dr Brian Oakley, SRC Secretary.

The Appleton Laboratory is responsible for managing most of the UK's scientific space programme, which includes satellites, balloons and rockets, for scientists working in universities. The Rutherford Laboratory has always been the home-based focus of the UK effort in high energy physics at CERN. With the closure of its particle accelerator, Nimrod, earlier this year, however, it has had to diversify. It now houses a central laser facility, computing facilities and is building a central spallation neutron source.

The SRC hopes that two activities in particular, space work and radio-communications, will benefit from the amalgamation of skills. At present work on both of these is done mainly at the Appleton Laboratory, although Rutherford has provided some engineering support for space projects in the past. Most recently, it helped design the stratospheric and mesospheric sounder for NASA's Nimbus-G satellite, launched towards the end of October. And it is currently working on the design of a millimetre wave radiotelescope with Appleton.

However some of the scientific staff at Appleton are far from content with the move. "The SRC came to a hasty decision without considering in

sufficient detail all the points we would have liked them to" said Dr Jo King, Chairman of the staff side at Appleton. He feels there is little advantage to be gained from the move. Usually, he says, satellites are built in several different places and put together in one place. This would still have to happen even when the two laboratories merge. In the case of radio propagation work, Dr King claims that there will be too much radiointerference at Rutherford to make such work effective. "We are making very sensitive radio receivers" he says "and being asked to put them in one of the noisiest places in the UK".

The move is expected to take five years to complete. Appleton will be receiving data from the UK Ariel 6 satellite, to be launched next year, and the UK/NASA/Dutch satellite, IRAS, which will be launched in 1981. Final transfer to Rutherford therefore cannot be completed until those satellites cease to be operational. The bulk of the move will take place between 1980-83.

The engineering support at Appleton is weaker than that at Rutherford, according to Dr Geoffrey Manning, Rutherford's deputy-director, because it is the smaller of the two. When they are amalgamated, he says, it should be possible to provide laboratory support for all of the UK's space programme at one site. According to another SRC official, this should maximise the chances of the UK participating with the United States in the multi-mission refurbishable satellite (mrs)—a satellite proposal which has gained much support from the astrophysicists but little enthusiasm from geophysicists.

Judy Redfearn

Greater powers for Dutch science minister

THE Dutch minister for science policy, Dr Marius Peijnenburg, has recently extended his role beyond that of co-ordinator. Together with one or two other ministers he now steers scientific research in government institutes and industrial laboratories. So far this has been the government's task.

With the minister for education and science, Peijnenburg will now be involved in decision-making at an early stage in university research as well as that carried out in the institutes of the Royal Netherlands Academy of Sciences. He will also be involved in formulating policy for the energy research centre, the aviation and space laboratory and the institute for aircraft development, which until now came solely under other ministers. Peijnenburg will also control TNO, the

national organisation for applied research in the natural sciences. It has a staff of about 5,000 and an annual budget of £55m.

The 1979 research budget totals around £1.5 billion, 2.05% of the gross national product. More than £0.7 billion comes from government and £0.8 billion comes from industry (mainly from Philips, Shell, Unilever, Dutch state mines and AKZO). In 1978 and 1977 the research budget was £1.4 billion and £1.2 billion respectively. For the first time, the minister for science policy is to have his own small budget for stimulating new projects in areas such as systems analysis and technology assessment. It should increase to £3m a year by 1981.

In 1979 the time spent on non-nuclear research as a percentage of

that spent on all energy research will increase from 36% in 1977 to 45% in 1981. Of the £57m spent on energy R&D in 1978, £30m is being spent on nuclear energy. Holland contributes £14m to the EEC energy research programme of which about £10m is spent on nuclear research.

Before the budget was published at the start of the new parliamentary year, the ministry for science policy announced that the Science Information Bureau will become permanent and be expanded slightly. It has been operating on a trial period for two years at the Academy of Sciences. And

at a meeting of the special parliamentary committee for science policy at the end of October, the minister announced the setting up of a group of experts to study the effect of microelectronics, especially microprocessors, on society. The group should report within three months. **Casper Schuurig**

Poland tries the taste of space

During 1978, three cosmonauts from Eastern Europe visited the Soviet Salyut-6 orbiting space station. **Vera Rich** talks to the Polish cosmonaut, Miroslaw Hermaszewski in Warsaw

MIROSLAW Hermaszewski, like his two brothers, is a pilot by profession, not a scientist. However as a cosmonaut he was obliged to fulfil a dual role—crew member and scientific worker. In the latter capacity Hermaszewski was responsible especially for space medicine, which he describes as “a Polish speciality”.

The medical programme concentrated on two experiments: “kardiolider” and “smak”. The first monitored the cardiovascular system “to determine the potentialities of the heart in space and also during pre-flight training”. This, Hermaszewski told me, meant that “from the psychological point of view we felt very safe, knowing our hearts were being constantly monitored, especially when we were not in contact with Earth”. (Constant contact, he explained, is not maintained as a rule, although Soviet ships around the world are on constant emergency watch.)

The second medical experiment “smak” (Polish for “taste”) was designed by the Academy of Military Aviation Medicine to monitor sensory changes under conditions of weightlessness. A few days before take-off, the cosmonauts sampled various menus and chose those they wished to take into orbit. “We chose the meals we preferred” said Hermaszewski “but after a few days in space we didn’t like them. This means that our sense of taste had changed.”

Challenged that the falling off of appreciation could be due to deterioration of the rations and/or boredom, Hermaszewski explained that the meals were specially prepared to preserve quality and taste, and also that there was “a menu-cycle of six days”. “And even then, it wasn’t exactly the same meal”, he added. “I mean, if on one day we had, say, *barszcz* and turkey at one meal, then six days later we’d have *barszcz* and turkey again, but not at the same meal.” The scientific team who worked out the experiments had, he said, developed a numerical method of estimating taste, which allowed the activity of the taste-buds to be monitored quantitatively.

Both “smak” and “kardiolider”, added Hermaszewski, were important for medicine in general. Versions of kardiolider were now being produced for use in post-coronary rehabilitation programmes, while “smak” could provide valuable data about sensory processes.

With the third main Polish experiment, ‘Syrena’, Hermaszewski clearly felt his responsibilities considerably. Although he worked in the experiment with the Soviet cosmonaut, Ivanchenkov—one of the long-stay Soviet crew—he was aware that the experiment had been developed by Polish scientists. “It was absolutely Polish from the beginning, and was called ‘Syrena’ after the symbol of Warsaw”. He admitted that during the running of the experiment which took 5 to 6 hours each time, he kept getting up at night to check the equipment (apparently quite unnecessarily).

‘Syrena’ was in fact an experimental capsule, used in conjunction with the Soviet ‘splay’ furnace, to produce highly homogeneous tellurium cadmium and telkurium/mercury alloys. Such alloys, of substances with very different molecular weights, are of considerable interest in semiconductor technology, and, in fact, both the Czech and East German cosmonauts carried out analogous experiments. It appears from Hermaszewski’s description, however, that although the Soviets provide the furnace, the various Comecon nationalities are responsible for the contents of the individual capsules and for working out the results—as well as for the symbolic code-name under which the experiment is performed.

This led, naturally, to the whole question of Comecon participation in manned space-flight. Hermaszewski stressed that, although he only knew the Czech and East German cosmonauts well—the other future crew-members arrived only towards the end of his own training—he felt confident that all the countries concerned (even Mongolia and Cuba) would have their own “concepts” to contribute to the programme. “But obviously that’s their secret for the moment.” He



Two faces of Cosmonaut Hermaszewski; left—meeting the press; right, set for space.

stressed, moreover, that there are a number of joint experiments in the programme, for example, some of the photographic surveys, and such things as the joint Polish-Soviet experiment on heat level.

As for Poland’s future plans in space, he was noncommittal. It was beyond his competence, he said, to comment on the future of the *Interkosmos* manned programme, when the present series of joint flights is complete, or on the possibility of wider international cooperation in space, perhaps on a UN basis. He stressed, however, that “We are only at the beginning of the Polish space programme.”

Fields of special interest for the future included: “cosmic technology (‘Syrena’ was an example of this); space physics; and experiments with some immediate effect on our economy — telecommunications, medicine, biology and so on”.

As for his own future, Hermaszewski said that he is now doing a great deal of work with young people, including a special TV programme. “I have had a great many letters from the young people of our country, and they are all very proud that Poland has begun the peaceful exploration of space!” □

US slashes funds to WHO and other UN agencies

In a pre-recess move that caught administration officials off-guard, the US Congress has made reductions totalling over \$27 million in next year's contribution to a number of key specialist agencies of the United Nations, including the World Health Organisation (WHO), the Food and Agricultural Organisation (FAO), and the United Nations Educational, Scientific and Cultural Organisation (UNESCO).

Furthermore the Congress has decreed that none of the remaining \$200 million contributions to these agencies and the UN itself may be used for the purpose of "technical assistance". The agencies are now trying to work out whether they can accept contributions under such conditions; if not, then 25% of the budget of each which is currently provided by the US, has been placed in jeopardy.

Hardest hit has been WHO, to which the US contribution has been reduced by 43%, or \$20.9 million; if this cut remains standing, the net result will be a 10% reduction in the organisation's total budget. Other cuts are: FAO reduced by \$3.2 million (10.7% of the US contribution); UNESCO by \$625,000 (2.1% of the contribution); World Intellectual Property Organisation by \$110,000 (100% of contribution); and the UN itself by \$2.85 million (2.5% of total contribution).

The decision to cut the contributions resulted from floor amendments accepted during debates in both

the House and the Senate on the budget of the State Department, from which such payments are made. President Carter was unwilling to veto the whole bill, but while signing it into law he said that the amendment "compromises this government's ability to fulfil its legally binding financial obligations".

The President added that he intended to recommend the prompt restoration of the technical assistance funds and eliminate the restrictive language. Administration officials said last week that it was proposed to do this with a supplementary appropriations bill that will be presented as soon as the new Congress meets in January. However, even if the language is changed, it is unlikely that the money will be made available in time to meet the next payments to the agencies, due in February.

The cuts reflect growing concern by some of the more conservative members of Congress over contributions made by the US to international organisations over which they have minimal control; unlike "voluntary" contributions to institutions such as the World Bank, the size of which is related to voting power in the institution, the specialised UN agencies, to which contributions are assessed broadly on grounds of the ability to pay, operate on a one-country-one-vote principle.

"Voluntary" contributions come under the foreign aid bill; and this

year Congress has attached to the bill conditions that prohibit its money being spent in countries such as Vietnam and Cuba of whose political regime it disapproves. "Assessed" contributions, however, although paid for out of the State Departments budget, are determined by international agreement, they are not supposed to be subject to the same type of pressures.

In previous years, attempts by members of the Senate to delete the technical assistance money from the department's appropriations and to transfer it to the foreign aid bill have been successfully resisted by the House. This year, however, caught up in the pre-recess rush of legislation, the House voted by 191 to 143 not to reject an amendment added by Senator Jesse Helms, whose cuts and restrictions subsequently passed into law.

"The use of assessed contributions to fund technical assistance is a device to tax the developed nations for the benefit of the undeveloped nations without proper consent from the developed nations", Representative John H. Rousselot, a former official of the right-wing John Birch Society, told the House in supporting the Helms amendment.

"It is changing the very nature of UN agencies away from programmes beneficial to all member nations and towards programmes of special interest to particular areas of the world."

David Dickson

Denmark expands research on Ante-natal diagnosis

THE Danish government has taken a significant step towards including ante-natal genetic diagnosis in the general health service. It has approved a plan for expanding laboratories and research centres involved in ante-natal diagnosis and last month granted funds for making it available to all 'risk patients' within approximately five years.

In 1972 the Danish Medical Research Council (MRC) decided to support and coordinate the work of several laboratories on congenital deformities and metabolic diseases. In 1975 the Ministry of the Interior (MI) appointed a committee to investigate the extent to which ante-natal diagnosis should be developed in Denmark, to outline how it should be developed and, finally, to investigate its economics.

The MI-committee presented its report (MI-report 803/1977) in February 1977. It concluded that the community would gain considerably in both human and economic terms if ante-natal diagnosis was given high priority in the general health service.

The report considered chromosomal

diseases and inbred metabolic disorders. The MI-committee estimated that about 4,000 chromosomal analyses would be needed yearly to check all risk groups—pregnant women older than 35 and those who have previously given birth to a child with a chromosomal disease. It recommended that seven centres specialising in amniocentesis—which is necessary for chromosomal analysis—be established. At present about 1,000 such analyses are done yearly.

Inbred metabolic disorders are very rare and diagnosis of each illness within this broad category requires its own special technique. Nevertheless, the report mentions several independent calculations which have found that for several specific diseases the benefit of ante-natal diagnosis and abortion in the case of positive results outweighs the cost. The main difficulty, however, lies with identifying those at risk. Either the whole population or selected groups can be screened. At present the Danish population as a whole is screened for phenylketonurea. The MI-committee has recommended that population

screening is now extended to include congenital myxodema.

Any further expansion of population screening for metabolic disorders must await the results of additional research. The MI-committee recommends, however, that programmes for selective screening should be incorporated into a few genetic research centres now. Centres should be established in Copenhagen and Aarhus, it says. They should coordinate all ante-natal biochemical diagnosis in Denmark. Six million kroner should be enough for about 2,000 such analyses a year—the current estimate of the need.

A communication from the National Health Service to all Danish hospitals, medical doctors and midwives marked the initiation of the new programme on 1 October. All pregnant women in Denmark belonging to a risk group are now offered genetic counselling and diagnosis. At the same time the financing of most prenatal genetic diagnosis will pass from the MRC to the general health service.

Sven Erik Godtfredsen

news in brief

● Explosion hazard at Windscale:

An unexpected and potentially dangerous build-up of hydrogen in one of the storage silos at the Windscale reprocessing plant has forced British Nuclear Fuels Ltd to close the plant temporarily. The build-up of hydrogen was discovered on Tuesday, 3 October. Safety procedures were instigated to bring it quickly under control.



Hydrogen is produced by a reaction between the magnox cladding material that is stripped from irradiated fuel elements before they go into the reprocessing plant, and the water in which they are stored in the silos. The hydrogen build-up was caused by a sudden increase in the temperature in the silos, due to the settlement of the magnox; this speeded up the reaction.

The temperature was lowered when the stand-by cooling system was switched on. The hydrogen was dispersed by forced air ventilation of the silos, but this in turn caused a discharge of radioactivity into the area around the silo. Although the discharge was within the safety limits, people working in the area had to be evacuated and decanning (pictured above) and reprocessing procedures halted.

● **Austria decides against nuclear energy:** Austria's new nuclear power station at Zwentendorf near Vienna may never be used, even though it is already loaded with the necessary radioactive elements. This follows a referendum on nuclear energy in which the Austrian people voted by a small majority against the use of nuclear energy in their country. In the poll, 50.5% of the votes were opposed to nuclear energy, and 49.5% in favour.

The referendum puts the Austrian Chancellor, Dr Bruno Kreisky, in a difficult position. The Zwentendorf power station forms a major part of the government's energy policy, and the ruling Socialist Party now has to decide what to do with it. It could be converted to a conventional power station. Dr Kreisky, who has described the referendum result as "a personal defeat", has however announced that he will not carry out his threat to resign if the vote went against atomic energy.

● **"No scientific barriers to nuclear waste disposal" says US report:** Despite limitations, existing scientific knowledge and technology is adequate to proceed with selecting and characterising sites for the permanent disposal of radioactive waste, according to a report issued last week by the US Department of Energy.

The report says that the successful isolation of radioactive waste from the biosphere "appears feasible for periods of thousands of years" provided that a systems approach for selecting the geologic environment, repository site and waste-form is utilised rigorously.

And it says that a broad range of underground media, such as salt, basalt, granite and shales, should be considered to provide a "conceptual framework" for program planning and establishing near-term priorities.

The report was prepared by an inter-agency review group chaired by Dr John Deutch, director of the office of energy research. After 30 days for public comment, a revised version will be prepared for the President as the basis for an administration policy on the handling, transportation and storage of nuclear wastes.

It recommends that an executive planning council be set up as part of a partnership between the federal government and individual states to plan and implement the nuclear

waste programme. And in order to assist in the progression to high level repositories, the report suggests that one or more intermediate facilities be established in several emplacement media and geologic environments "at the earliest practicable date." If an alternative to salt is selected for the first high-level waste depository, this is unlikely to be ready until 1992 at the earliest, the report says.

● **US and China agree to exchange research workers:** China is expected to send over 500 postgraduate students and research workers to the US over the next year under the terms of a verbal agreement made in Washington last week between the National Science Foundation and a visiting delegation from the People's Republic of China.

The most popular subjects are likely to be physical and biomedical sciences, engineering and applied technology. In exchange about 60 US researchers will visit China, although primarily to study subjects in the social sciences, language and literature, and archaeology and art.

In addition to these exchanges, many more Chinese students will come to take courses in the US through private arrangements with US universities; the first of these have already started courses at Stanford University and the University of California at Berkeley.

Under the terms of the agreement, which was reached after a two-week visit by the delegation which had included 14 universities and colleges, each side agreed to use "its best efforts" to fulfil requests for study opportunities, and to pay the costs for its participants.

● **Lund looks at UNCSTD:** How are preparations for next year's United Nations Conference on Science and Technology for Development (UNCSTD)? "Rather bad but not disastrous", says Docent Jon Sigurdson, director of the University of Lund's Research Policy Program and co-editor of the Lund Letter, circulated regularly to governments and people interested in UNCSTD. "There are organisational problems, and if the organisation doesn't work well we can't pinpoint the questions that should be discussed."

It is well known amongst officials working with UNCSTD matters that the UNCSTD Secretariat and the UN Office for Science and Technology have found cooperation difficult, with each one trying to make the conference its own show. Similar problems have arisen between the Secretariat and various UN agencies—UNESCO, for example—and UNCSTD's Secretary-General Da Costa, who has been on sick leave for extended periods, has not been able to provide the strong leadership necessary to bring the squabblers into line.

"The first phase of the preparations—the national analyses leading up to the national papers—is nearly over now", says Mr Lennart Båge of the Ministry of Foreign Affairs. "We are now anxiously waiting for the Secretariat to analyse these papers. As the third preparatory committee meeting has been postponed until January, we need good background documentation in good time for the meeting to make it an efficient starting-point for the international negotiations leading up to the conference."

The Research Policy Program is itself about to begin going through the sixty or so national papers to hand to see which themes they have taken up and where their deficiencies lie. "From what we have seen", says Docent Sigurdson, "most national papers seem to have dealt only with item one on the conference's agenda: science and technology for development. Very little attention seems to have been paid to the other three items, especially the last on: science and technology for the future".

news and views

Hormones and cell culture

from Philip S. Rudland

It is rare in science these days for a conference to be convened in memory of any one person, but such was the high regard in which Gordon Tomkins was held that the Sixth Cold Spring Harbor Meeting on Cell Proliferation* was convened in his memory in a field in which he was one of the father figures, that of Hormones and Cell Culture. Gordon Tomkins was one of the first to consider that animal cells in culture could be used almost like bacteria for following molecular events, and in particular the effects of hormones on various cell functions. Nowadays the Hormones and Cell Culture field seems to be split into two camps, one concerned with the isolation of growth factors and their mode of action on cell proliferation and the other with the action of hormones in inducing cellular differentiation.

Cell proliferation

On the cell proliferation side R. Iglesias (Santiago) emphasised the historical importance of hormones, particularly oestrogens, in inducing tumours in animals, although it is uncertain whether oestrogen usually affects cell proliferation directly as reported for a human breast cancer cell line (M. Lippman *et al.* National Cancer Institute, Bethesda), or through intermediary growth factors (estromedins) proposed and identified by D. Sirbasku *et al.* (University of Texas, Houston) as necessary for the growth of cells from some oestrogen dependent tumours *in vivo*. The major mitogens for cultured cells, the so-called growth factors, seem to be becoming purer and the purification of one from platelets was extensively discussed (R. Ross *et al.* University of Washington, Seattle; C. Heldin *et al.* Institute of Medical and Physiological Chemistry, Uppsala). A new growth factor of less than 3,000 daltons has been isolated from bovine pituitaries; this factor specifically stimulates the growth of a rat mammary epithelial

cell line from a hormone-dependent transplantable rat mammary carcinoma (T. Kano-Sueoka *et al.* University of Colorado, Boulder). Vasopressin could act like a growth factor in 3T3 fibroblastic cells (H. Rozengurt, Imperial Cancer Research Fund, London). The importance of hormones and growth factors in promoting cell proliferation was emphasised when certain of these agents were reported to support the growth of several different cell lines in the absence of serum (J. Bottenstein *et al.* University of Calif. San Diego; Serrero *et al.* SPNCI, Nice; Lippman *et al.*). Cultured cells and tumours are also proving a rich source of growth factors. The discovery of growth factors was stimulated to some extent by the hypothesis that tumour viruses produced their transforming action on cells, at least in part, by the production of endogenous polypeptide growth stimulatory factors (G. Todaro, National Cancer Institute, Bethesda). Todaro now reported that a human fibrosarcoma released growth-polypeptides which could interact with receptors which normally bind a serum growth factor, MSA. Conversely however, normal rat or hamster liver released growth-promoting activity for virally, spontaneously and chemically transformed cells but which did not stimulate their normal counterparts (A. Lipton *et al.*, Hershey Medical Centre, Hershey).

Before discussing the action of growth factors further one needs to know how and where they act in the cell cycle. A. Pardee (Sidney Farber Cancer Institute, Boston) compared two different classes of model for the cell cycle which could account for the variability in the distribution of cellular intermitotic times, deterministic Gaussian types and random or probabilistic types. He suggested that a deterministic model giving a reciprocal normal distribution could be made to fit the daughter cell generation times as well as the semilogarithmic or first order plots of the probabilistic model, in contrast to a recent paper (Shields

Nature 273, 755; 1978) which claimed the contrary. However it seems that the choice between the two types of model at least is deterministic, depending on which side of the Atlantic the decision is made. Two slightly different versions of how growth factors affect the cell cycle were presented. First C. D. Stiles *et al.* (Sidney Farber Cancer Institute, Boston) considered that one growth factor, platelet factor, 'committed' quiescent 3T3 fibroblastic cells to a process which eventually led to a first order increase in the rate of cellular entry into S phase, while platelet-poor plasma was required to allow the cells to 'progress' to the increased rates of DNA synthesis. However L. Jimenez de Asua *et al.* (Friedrich Miescher-Institut, Basel) considered that the growth factors fibroblast growth factor and prostaglandin $F_{2\alpha}$ delivered both signals, one to commit the cells to change their growth rates and the second to increase the rate of entry into S. Hormones such as insulin and glucocorticoids alter only the second response.

Where do growth factors act?

The biochemical as opposed to the kinetic mode of action of growth factors is also unclear. Two models have arisen; the earlier envisaged that growth factors bind to cell surface receptors and then trigger intracellular 'messengers' without entering the cell; the more recent model considers that the growth factors are 'internalised' and go to 'control sites' inside the cell. Support for the earlier model was provided by the carefully controlled experiments of D. Cunningham *et al.* (University of California, Irvine) with the protease thrombin. He has found that this can still stimulate chick embryo cells' division even when attached to Sepharose beads which cannot enter the cell, although unattached thrombin was internalised (J. Quigley

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*Held on 29 August–3 September.

et al., State University of New York). A series of speakers starting with S. Cohen (Vanderbilt University, Nashville) showed that epidermal growth factor (EGF) could be internalised and eventually degraded in lysosomal vesicles. Whether internalisation of EGF is required for mitogenic activity, however, is unknown, although C. Fox (University of California, Los Angeles) found that the concentration of EGF required for half-maximal binding to the surface receptor was much less than for internalisation. The latter corresponded more closely to the concentration required to stimulate maximum rates of DNA synthesis, which suggested that internalisation might be the limiting step. Internalisation was not a unique property of EGF, however, since thyroid-releasing-hormone (A. Tixier-Vidal *et al.* Collège de France, Paris) and melanocyte stimulating hormone (A. Lerner *et al.* Yale University) are also internalised. If the first model for the action of growth factors is correct, there is a requirement for second messengers or a chain of messengers to continue the 'growth stimulus' inside the cell. A much simpler example of such a system is that of insulin control of a single enzyme, glycogen synthetase, in rat adipocytes (J. Lerner *et al.* University of Virginia, Charlottesville). Two separate biochemical mechanisms were proposed to control a multiply-phosphorylated enzyme, glycogen synthetase. Phosphorylation of this enzyme and hence its activity were then controlled by a cyclic AMP-dependent protein kinase, a cyclic AMP-independent kinase and by the calcium-stimulated action of phosphorylase *b* kinase. Models of the first type for the action of growth factors may then involve biochemical pathways of nightmarish complexity.

Differentiation

Studies of the effects of hormones on differentiation processes emphasised the reciprocal nature of proliferation and differentiation. Thus in cultured adrenocortical cells, fibroblast growth factor inhibited steroidogenesis, the differentiation response, while ACTH, which stimulated steroidogenesis; inhibited cell proliferation (G. Gill *et al.* University of California, San Diego). The evidence that ACTH acts through cyclic AMP in this case was good since Y-1 adrenal cells could be selected which were deficient in some component of the adenylate cyclase in cyclic AMP-dependent protein kinase activities and these also failed to respond to ACTH (B. Schimmer *et al.* University of Toronto). Different inducing agents could also commit more primitive tissue culture cells (stem cells) to a new cell type. Thus vitamin A induced the formation of endodermal

cells from mouse embryonal carcinoma cells *in vitro* (S. Strickland, Rockefeller University, New York), and dimethylsulphoxide (P. S. Rudland *et al.* Imperial Cancer Research Fund, London) and other agents (J. Lever, Salk Institute, San Diego) which induced Friend erythroleukaemic cells to synthesise globin, caused a rat mammary carcinoma stem cell line to differentiate into alveolar-like secretory cells. Interestingly the effect of hormones on the responsive cell in the animal may not necessarily be direct, but through other cell types. Thus during embryonic development the mammary rudiment of the male mouse regresses as a result of androgen action, not on the epithelium, but on the surrounding mesenchymal cells which in turn causes the epithelium to become necrotic (K. Kratochwil *et al.* Österreichische Akademie der Wissenschaften, Salzburg).

The majority of the work on hormonal control of differentiation at the molecular level was concerned with the modulation of amounts of intracellular enzymes and secreted hormones in cells which were already differentiated, rather than changing the cell to a new type as above. Hormones mainly exert their effect on specific gene expression by increasing the amount of specific cytoplasmic mRNAs. Moreover in spontaneous rat hepatoma cell variants

the control of the production of more than one gene product occurs at different, unconnected steps beyond the interaction of the glucocorticoid with its cytoplasmic receptor (E. Thompson *et al.* National Cancer Institute, Bethesda). In a *tour de force* of molecular biology J. Baxter *et al.* (University of California, San Francisco) first produced a working hypothesis that glucocorticoids rapidly modify chromatin structure (as measured by the binding of *E. coli* polymerase) in a gross way that nevertheless affects the expression of only a few genes in rat pituitary (GC, GH₃) cells, notably the growth hormone gene. He then reported the nucleotide sequences of the DNA complementary to human and rat growth hormone and human placental lactogen mRNAs, which were remarkably similar, and finally showed that a new protein containing immunoreactive growth hormone sequences could be obtained in bacteria by ligating cloned rat growth hormone cDNA and the ampicillin resistance gene of another plasmid. Thus the conference had come full circle from Gordon Tomkin's original belief in that a hormonally-regulatable gene from cultured animal cells could now be expressed in a bacterium. The conference ended with a highly enjoyable jazz session, a fitting tribute to Gordon Tomkins, the jazz musician. □

Antenatal diagnosis of genetic defects

from Bob Williamson

A NEW and most exciting method of antenatal diagnosis is reported in the *Lancet* (ii, 910, 1978) by Y. W. Kan and Andree Dozy, of the San Francisco General Hospital. They have applied recombinant DNA techniques developed to study the organisation of the human globin gene family, to diagnose successfully a fetus at risk for sickle cell anaemia. The method is very sensitive and safe and will no doubt be applied immediately in similar cases. However, the implications are far wider, for this result promises that any diseases for which a gene-specific probe can be identified may well be diagnosed in the same way.

As readers of these columns know, the arrangement of genes for which specific probes are available can be studied by restriction enzyme analysis to provide a detailed physical map of the coding and surrounding sequences.

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The human β globin gene occurs only once in the haploid genome, is separated from the related gene coding for the globin chain by some 7,000 base pairs, and contains at least one intervening sequence several hundred base pairs long. The map of restriction sites round the gene has been constructed by several groups, and there is broad agreement on its appearance (see *News and Views* 276, 13; 1978). Kan has now shown that for people of white, black or Asian origin with no haematological disorder, there are usually sites for the restriction enzyme *HpaI* generating a fragment 7.6 kb (kb=1000 bases) long and including the β globin gene. This was always the case for the whites and Asians tested. Blacks with sickle-cell anaemia have almost invariably lost the 3' *Hpa* site, and a new one, which gives rise to a fragment 13 kb long is found instead. (Another restriction site giving a 7.0 kb β globin gene fragment is also occasionally found but this seems to be a polymorphism among blacks unrelated to sickle-cell anaemia). Natur-

ally, in general the cases with homozygous sickle-cell disease had a 'double dose' of the 13.0 kb fragment while heterozygous carriers had one 13 kb fragment and either the 7.0 or 7.6 kb fragment.

Sickle cell anaemia is due to a single base change in the DNA causing a substitution of valine for glutamic acid in position 6 of the β -globin protein sequence; this change presumably has nothing to do with events thousands of base pairs away. What seems to have happened is that the sickle cell mutation, which confers resistance to malaria in heterozygotes, just happened to occur in an individual who had either lost the site for *HpaI* at 7.6 kb, or whose genes had undergone some rearrangement at that site. Then the rearrangement or change far away from the globin gene was 'fixed', to be locked in linkage to it in subsequent generations.

The fact that a second linked polymorphism has been found (and several have been suggested for the other globin genes as well) argues that these are rare but by no means unknown events. They may not occur often at any given site, but there is a likelihood (if one can generalise, as usual, from the globin genes to other structural genes) that several could be identified within a few thousand base pairs of any gene. Any mutation thus carries with it a characteristic detritus of surrounding gene material, in arrangement and sequence. This in turn implies that an advantageous mutation becomes 'fixed' more rapidly than random mutational drift occurs in unselected sequences.

One of the most remarkable aspects of Kan and Dozy's result is the sensitivity of the method. Only 15 ml of amniotic fluid were taken. There was sufficient DNA in the foetal cells suspended in this sample of fluid to allow direct analysis without further cell culture. DNA from the parents' white blood cells, and from any siblings, must also be prepared, to ensure that the linkage is valid in the case under study. Hybridisation and autoradiography take only a week, allowing a result to be obtained earlier than with methods that require a foetal blood sample, which must be taken later in the pregnancy. Termination can be offered in good time, and the investigation has none of the technical risks associated with taking a foetal blood sample, and does not require such sophisticated equipment.

By recombinant DNA techniques, the entire human genome can be easily cloned in lambda phage or in plasmids; at least four groups have done so already. These 'clone libraries' are being made available to groups wishing to study particular diseases and who have developed gene-specific probes. Consider, for example, identifying a similar

linkage group for a condition such as haemophilia. Polysomes making factor VIII protein could be precipitated with antibodies, the RNA isolated and a DNA complementary copy cloned in a plasmid. This can be used to hunt for the gene sequence, and polymorphisms identified—attempts would be made to link these to the haemophilia mutation. If this can be done (and for a recessive illness, there are good theoretical reasons to think that it can) there is no need to look at the very small sequence change, often involving a single base pair, causing the disease—linkage is enough for antenatal diagnosis. Another advantage of the method is the ease of diagnosing heterozygotes—they will have one, but not both, genes affected—a pattern easily seen using restriction enzymes and hybridisation.

Several diseases will be candidates for this approach in the months ahead. Some cases of β -thalassaemia do not involve a gene deletion, but could be linked in a similar way to specific restriction sites, particularly where family

studies are possible. It should be easy to develop gene-specific probes for single gene defects where the affected protein is known, such as agammaglobulinaemia, Tay-Sachs disease and Lesch-Nyhan syndrome. Even conditions where the protein affected is not yet identified, such as Duchenne muscular dystrophy, might be studied using recombinant DNA in this way.

Since it is often thought that this technology increases the overall abortion rate by making more knowledge available to parents, perhaps it should be noted that Kan and Dozy's case turned out to have the heterozygous sickle cell trait, was not terminated, and will grow up haematologically normal. Far from leading to more terminations, the availability of accurate antenatal diagnosis for severe hereditary disease should allow a decrease in abortion, since parents can continue a pregnancy knowing that a child will be healthy rather than terminate all pregnancies for fear of a possible severe illness and death. $\square$

Positrons at the centre of the Galaxy

from J. J. Quenby

AFTER a long struggle, balloon experiments seem to have established the presence of detectable γ -radiation due to positive electrons at the centre of our Galaxy. Whether this radiation is ultimately telling us about the remains of supernova explosions, the cosmic ray distribution, the physics deep inside the magnetic fields of pulsars or black holes remains a largely open question.

Positrons can react either in flight or at rest with electrons, bringing about their mutual annihilation and the production of two gamma rays at an energy of 511 keV. If the annihilation is at rest, a sharp 'line' flux of photons results but if the interaction is in flight, Doppler broadening of the feature spreads out the apparent frequency of photon emission. A sharp line is the characteristic signature sought by balloon-borne detectors used in γ -ray astrophysics. Even though the positrons responsible may be very slow, it is not necessary for all the annihilation radiation to produce a line. At low matter densities, normal in astrophysical situations, positrons and negative electrons may form a bound state known as positronium. 75% of all annihilations may occur in a 3-photon decay of this state, producing a wide spread of photon energies.

The earliest indication of line radia-

tion from the galactic centre was obtained by Johnson, Harden and Haymes (*Astrophys. J.* **172**, L1; 1972) using a crystal scintillator with a resolution of ± 24 keV. This group later improved their technique getting a resolution of ± 11 keV (Haymes *et al.* *Astrophys. J.* **201**, 593; 1975). Both of these results gave line positions more than one standard deviation away from the 511 keV energy. A more definitive study has now been reported by Leventhal, MacCallum and Stang of Bell and Sandia laboratories (*Astrophys. J. Lett* **225**, L11; 1978) using a rather difficult technique involving cooled germanium detectors. A resolution down to 3 keV is achieved by keeping the solid state detector at around 100 K or less. One problem encountered by these workers was the instrumental background line at 511 keV due to cosmic ray interactions. However, by pointing on and off the source, a galactic centre 511 keV line flux of 1.2×10^{-3} ph S^{-1} cm^{-2} was found, together with some probable spreading due to the additional 3-photon decay mode. Against this confirmation of the Haymes group's work is a statement from the satellite experimenters on HEAO-1 that as yet no line flux around 500 keV is seen (HEAO-1 Newsletter 2, April 1978). Here again only a crystal detector with broad band energy resolution was used.

What explanation can be advanced

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for the striking Leventhal *et al.* result? Positrons may occur deep within the magnetic field regions of pulsars, close to the surface, due to energetic proton reactions or indirectly from electric field acceleration of electrons. However, the strong gravitational field might be expected to significantly shift the line frequency from the 511 keV level.

For a long time it has been noted that radioactive decay products arising from nuclear burning at the time of supernova explosions may produce observable γ -ray lines (for example, Colgate, *Astrophys. Space Sci.* **8**, 457; 1970). The explosion is preceded by a silicon burning process in which reactions with free α -particles leads to the synthesis of elements near iron in the periodic table. In particular the decay of the ^{56}Co and the ^{44}Sc produced includes long half-line positron emission. Some fraction of the exploding star enters the interstellar medium. If the positrons come from this blown-off envelope, spatial and time variations are expected in the line flux.

Another approach to the explanation

is to consider the interactions with the residual medium experienced by energetic cosmic ray particles in the Galaxy. In particular, particles above 10 MeV can excite positron emission from C, N and O atoms. Ramaty, Stecker and Misra (*J. geophys. Res.* **75**, 114; 1970) have made detailed estimates based on this mechanism but demand a cosmic ray energy density more than 10 times that seen near Earth. One way out of this problem is to realise that explanation of the 50 MeV γ -ray flux from near the galactic centre first seen by OSO-III (Kraushaar *et al.*, *Ap. J.* **177**, 341; 1972) may require a similar cosmic ray density enhancement.

Further information on the line emission should come when HEAO-C is launched, carrying a cooled germanium X-ray detector and also in the more distant future, from a larger version of this apparatus on board the GRO mission. It is then that the theoretical possibilities may be sorted out and other more speculative sources of positrons such as evaporating primordial black holes may perhaps be brought into consideration. $\square$

Channels in membranes

from Venetia A. Saunders

THE latest applications of model membrane systems to the study of bacterial membrane function has provided an important insight into the role of the major outer membrane protein—matrix protein—and the mechanism underlying the action of the bactericidal colicin K protein.

The outer membrane of Gram-negative bacteria such as *Escherichia coli* is freely permeable to low molecular weight hydrophilic molecules such as glucose and amino acids (for a recent review see Di Rienzo, Nakamura & Inouye *A. Rev. Biochem.* **47**, 481; 1978). Recent results indicate that several outer membrane proteins have a role in the permeation of such molecules through the membrane.

Nakae (*Biochem. biophys. Res. Commun.* **71**, 877; 1976) has shown that incorporation of matrix protein (which corresponds to porin, molecular weight 36,500), a major outer membrane protein from *Escherichia coli*, into artificial membrane vesicles restores their permeability to a variety of saccharides. Such reconstituted vesicles show similar molecular sieving properties to those of the intact cell membrane. (Estimates of exclusion limits range from about 600 to 900 daltons

for saccharides). It has been tentatively proposed that one function of matrix protein is to generate aqueous transmembrane channels that permit the rapid diffusion of small hydrophilic molecules. This notion is supported by the finding that mutants of *E. coli* lacking matrix protein are defective in passive diffusion (Bavoil & Nikaido *Molec. gen. Genet.* **158**, 23; 1977). There is also evidence (Schnaitman, Smith & de Salsas *J. Virol.* **15**, 1121; 1975) suggesting that matrix protein can be utilised as a receptor for the temperate coliphage PA-2 and that the amount of this protein is substantially reduced in lysogenised strains of *E. coli*. This could provide a mechanism for the exclusion of superinfecting phages. Ultrastructural studies of Steven and coworkers (*J. Cell Biol.* **72**, 292; 1977) have revealed that the matrix protein is present in a highly organised array within the architecture of the outer membrane: 10^3 copies of polypeptide per cell are arranged in a periodic monolayer which exhibits three-fold symmetry and triplet indentations (possibly corresponding to channels).

A recent paper from Schindler and Rosenbusch (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3751; 1978) now describes the successful integration of matrix protein into planar lipid bilayers and shows that the incorporated protein creates voltage-controlled channels

across these membranes. Those channels conduct ions and are freely permeable to small hydrophilic molecules. Analysis of the electrical properties of the membranes indicates that channels are induced irreversibly by voltage and that the number of channels is directly related to the matrix protein content of the membranes. Channels are proposed to be of uniform size, approximately 1 nm in diameter and to assume either an open (active) or closed (inactive) state. Schindler and Rosenbusch speculate that the closing of channels, which is favoured by high potentials, could be a mechanism for regulating permeability of the outer membrane. It is interesting to note that channel induction in the bilayers is voltage-dependent between pH 6 and pH 8, but is spontaneous at pH 10. The fraction of active open channels in bilayers at pH 10 is roughly equivalent to that in the bacterial cell, whereas the fraction of open channels in bilayers at physiological pH is significantly less. This discrepancy presumably reflects the inevitable artificiality of *in vitro* membrane systems.

In this issue of *Nature* (page 159) Schein, Kagan and Finkelstein report the use of planar phospholipid bilayer membranes to identify the precise mechanism of colicin action. They find that treatment of planar phospholipid membranes with colicin K also results in the formation of voltage-dependent ion-permeable channels, with the number of channels generated dependent on colicin concentration. These observations are consistent with the known effects of the E1 functional group of colicins (K, E1 and Ia) on the bacterial cell. They exert their bacteriocidal effects by a discharge of the 'energised state' of the cytoplasmic membrane and promotion of a net efflux of ions from the cell. One point of interest is that preparations of colicin K from *Proteus mirabilis* and *E. coli*, which are apparently specified by identical plasmids, differ somewhat in respect of their molecular weights and their quantitative effects on the artificial membrane system.

Schein *et al.* suggest that a single colicin channel could deplete a bacterial cell of all its potassium and other small ions within minutes. The relatively indiscriminate flux of ions through the channels could provoke at least partial depolarisation of the membrane. Since the energy to drive active transport systems derives from the transmembrane potential and the pH gradient (in accord with the chemiosmotic hypothesis), membrane depolarisation could in turn disrupt active transport. Indeed it is possible that depolarisation *per se* could lead to channel closure. The finding that E1 type colicins create voltage-dependent channels in artificial

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RECENT exciting advances in physics at millikelvin temperatures have been made possible by an ample and relatively cheap supply of helium-3 for operating dilution refrigerators, but it looks very much as though this happy situation is now coming to an end, with an impending world-wide shortage of the gas. The Radiochemical Centre at Amersham, for example, is not accepting any further orders: even when the present backlog of orders, some dating back to the beginning of the year, has finally been cleared, it is apparently by no means certain that further supplies will become available for sale.

One of the principal uses of helium-3 is as the working fluid of the dilution refrigerators which have become the work-horses of millikelvin research. The principle on which they operate is extremely simple, just depending on the cooling effect which takes place when liquid helium-3 is allowed to dissolve in liquid helium-4. The refrigerator is usually arranged so that the process is continuous, with provision being made for separating the two isotopes again in a still, and for recirculating the ^3He back to the mixing chamber through a series of heat exchangers in which it is cooled by the cold dilute mixture leaving the chamber. Optimising the design of such machines is quite a complicated exercise, but progress over the past few years has been rapid: the refrigerators developed in Grenoble by Frossati, for example, are now able to sustain temperatures of around 2.5 mK in continuous operation, which is lower by a factor of four than the best available commercial machines of only a couple of years ago.

The present helium-3 famine arises, probably, for three main reasons. First, there has been a decided increase in the number of research

Will detente kill millikelvin research?

from P. V. E. McClintock

groups building or buying dilution refrigerators, partly stimulated by the discovery of superfluidity in liquid helium-3 itself: all these machines require an initial charge of helium-3. Second, the relatively large machines which people are now tending to build require correspondingly large quantities of helium-3; this is particularly true of the Frossati designs, which incorporate exceptionally large diameter tubes within and between the lower heat-exchangers in order to reduce viscous heating effects as the liquid moves through the system. Finally, there is detente.

The ample supply of helium-3 enjoyed by cryophysicists in recent years has been produced as a byproduct of the nuclear weapons programme. The tritium used in thermonuclear devices decays through β -emission to helium-3, with a half-life of about 12 years. Although nobody seems very keen on discussing the exact arrangements made in practice for collecting the helium-3, one may infer that it is very much less convenient to do so once the weapon is in its final form ready for use, so that the ready availability of gas for sale to researchers and the cryogenics industry probably depends on continued manufacture.

Although the dilution refrigerator in its present form can certainly be regarded as a byproduct of the thermonuclear arms race, and any slackening of the latter must be warmly welcomed, it will be sad indeed if the excitingly rapid progress now being made in the millikelvin field is seriously curtailed. There are, however, two reasons to remain fairly

optimistic about the long term outcome.

First, one may note that after being set up with its initial charge of helium-3, very little of the gas need get lost from a dilution refrigerator, such losses as there are occurring only by leakage or through human error, both of which can be minimised by careful design. So, provided that there is a slowdown in the rate at which new machines are being commissioned, the present gap between demand and supply may eventually decrease again. Unfortunately there is no sign of this happening just at the moment.

Second, there always remains the possibility of extraction from natural helium-4, which usually contains a few parts in 10^7 of the light isotope. This certainly can be accomplished—at a price—and, indeed, was used as a small scale method of helium-3 production before the advent of extensive thermonuclear weapons programmes. There is a considerable variation in the helium-3 content of the gas from different wells. By choosing the right well and using modern isotopic separation techniques it just might be possible to produce helium-3 at a price not too much above the present £75 per S.T.P. litre. Furthermore, if the rumours about certain Polish gas wells prove to be correct—that they produce helium containing about 1 part in 10^5 of helium-3—a return to extraction from the natural helium could even lead to a reduction in the current price, at the same time making Poland the World's chief supplier of helium-3.

Until an alternative source of helium-3 is developed, detente is undoubtedly going to bring a measure of deprivation to the millikelvinists.

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membranes is likely to provide a novel approach to the exploration of membrane function. □

Complexity and stability

from N. MacDonald

THE relationship between the complexity and the stability of an ecosystem has been debated for some years by mathematically inclined ecologists, and has occasioned the choice of title for an extremely influential book (May, *Stability and Complexity in Model Ecosystems*, Princeton, 1973). A possible relation-

ship is indicated by a simple argument concerning a food chain, in which species A depends solely on species B, species B solely on species C, and so on. Any break in the chain eliminates all the previous members A, B . . . On the other hand, if there are alternative paths through a food network, one may expect the general structure of the network to survive the loss of one species. Here complexity is interpreted as the fraction of the pairs of species which directly interact, a quantity which may be termed connectance. Stability is interpreted as survival of all remaining

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species when one is lost.

As one makes the model more mathematically specific, so one has to be specific in the choice of a measure of complexity, and in the type of stability property that is studied. Some version of connectance, often allowing for an average over interactions of different strengths rather than simply counting the interactions, is usually the measure of complexity, in particular in a series of papers which appeared in *Nature* a few years ago. (Gardiner & Ashby, *Nature* **228**, 784; 1971; May, *ibid* **238**, 413; 1972; Siljak, *ibid* **249**, 280; 1974; Daniels & Mackay, *ibid* **251**, 49; 1974; Roberts, *ibid* **251**, 607; 1974; Gilpin, *ibid* **254**, 137; 1975; Saunders & Bazin, *ibid* **256**,

120; 1975.) Some criticisms of connectance as a measure of complexity can be found in another paper of this series (Somarjai & Goswami *Nature* 236, 466; 1972).

From a philosophical point of view one may argue that it is paradoxical to seek a single index to characterise complexity. As argued by R. Rosen (*Int. J. Gen. Systems*, 3, 227; 1977) the complexity of a system may involve the necessity of employing several parallel descriptions of the system. However connectance does pick out and quantify the intuitively satisfying notion that a collection of N solitary individuals is less complex than a collection of $N/2$ interacting pairs, and this in turn is less complex than a collection in which all the potential $\frac{1}{2}N(N-1)$ interactions take place.

The papers cited interpret stability as the stability of a steady state against small disturbances, that is to say they study the local stability of the equilibrium point of a dynamical system. Working with this narrow concept of stability the earliest of these papers showed that for components interacting at random, high connectance reduces the likelihood of stability. Later papers in the series incorporate restrictions on the interactions that seem obligatory if the system is to be acceptable as a model ecosystem. (For recent views of how this work may relate to observations on real ecosystems, see McNaughton, *Nature* 274, 251; 1964; Pimm & Lawton, *Nature* 275, 542; 1978.)

One way of going beyond local stability is by constructing Liapunov functions, which are analogous to the potential energy of a physical system. A recent book (Siljak, *Large Scale Dynamic Systems; Stability and Structure*, North-Holland, 1978) examines how the Liapunov stability of an ecosystem model is affected by the complete or intermittent removal of individual species. The criteria used for stability involve the diagonal dominance of interaction matrices. Thus it is required that individual populations are so stable in isolation that interactions with the other populations cannot destabilise. As the connectance increases, in the sense that interactions between species become stronger relative to the stabilising interactions within species, stability is lost. It is clear that the relevance of this to systems involving predation is remote. Some interaction between the predator species and at least one prey species is essential for survival of the predator. Another possible way of removing the restriction to local stability, with the implied reliance on linearisation of the model, is suggested by a recent paper in a rather different

context (Froeschlé, *Phys. Rev. A* 18, 277; 1978). The topic investigated is the relation between connectance and global behaviour of a certain set of formal transformations. The context of this work is the problem of the transition from integrable (periodic or multiply periodic) orbits to ergodic orbits in Hamiltonian systems, and in systems of formal transformations with area-preserving properties analogous to the Liouville property of Hamiltonian systems. Perhaps the most readable accounts of this problem are some reviews by J. Ford (for example in *Dynamical System Theory for Economists*, (ed. Szego) Springer-Verlag, in press).

Froeschlé takes N (up to 50) two-dimensional area-preserving mappings τ_i of a certain form and defines a multi-dimensional mapping T for which he can specify that T involves coupling between τ_i and τ_j for $j-i \leq N_0$. Thus N_0 , which is taken ≤ 10 , is the connectance. It turns out that there is a sharp increase, at about $N_0=7$, in the extent to which ergodic orbits predominate. It remains to make a connection between the transition to ergodicity and the onset of instability. In practice the

way in which one recognises the emergence of ergodicity is by computing the rate at which two orbits, initially very close, drift apart. This is linear with time for integrable orbits, but exponential for ergodic orbits. When using the local instability of an equilibrium point as the property to be interpreted as system instability, one is concerned with the exponential departure of a slightly disturbed orbit from that point. When one uses ergodicity as the relevant property, one is concerned with the rather more general feature of exponential separation of two initially close orbits. This would seem at least as good a candidate for 'the stability' of the system.

It remains to be seen whether the technical difficulties of investigating ergodicity allow one to work with systems satisfying the rudimentary requirements for a model ecosystem. Hamiltonian systems are well known, as discussed in May's book, to be unsuitable as model ecosystems. However it is now known, as reviewed recently in these columns (*News & Views* 271, 305; 1978; 274, 847; 1978) that behaviour analogous to ergodicity can arise in a much wider class of dynamical system. □

Informational suppression in higher organisms

from Peter Piper

MUTATIONS in one gene that correct specific defects in others can be isolated in many organisms and a knowledge of their mechanisms of action can throw considerable light on how various molecular components of the cell interact. A recent symposium on nonsense mutations and nonsense suppressors* reviewed ways in which an important class of these mutations, the so-called 'informational suppressors', partially correct certain defects in the protein-coding regions of structural genes by altering components of the protein synthetic machinery of the cell.

Mutations in the protein-coding regions of structural genes fall into different classes depending on the nature of the mutational change. In the deletion mutants a complete segment of the DNA is absent from the gene and this segment may include part of the protein-coding sequence. In another common class, missense mutants, a base substitution in the coding region of the gene leads to the substitution of one amino acid for another in the protein sequence. This alteration is selected on the basis of a change in the protein's properties, frequently a partial or complete loss of function, and can be corrected by the appropriate

tRNA mischarging whereby the aberrant amino acid is replaced by another that restores functionality. Such mischarging can be induced by suppressor mutations in tRNA or aminoacyl-tRNA synthetase genes.

Nonsense mutations produce a base change in the sequence of a gene such that one of the three termination codons of the genetic code, UAG, UAA or UGA, is generated within the protein-coding region resulting in the synthesis of an incomplete polypeptide gene product. They can be reverted by suppressor mutations that allow insertion of an amino acid in response to the nonsense codon. Similarly, frameshift mutations, which alter the reading frame of an mRNA by base insertions or deletions in the coding sequence can be reverted by suppressors that partially restore the reading frame during translation a short distance after it has been displaced by the frameshift mutation. Although nonsense and frameshift suppressors can be generated by mutation of genes for ribosomal proteins or tRNA modification enzymes, in bacteria and yeast where they have been biochemically characterised most of them arise by mutation of tRNA genes (J. D. Smith, MRC Laboratory of Molecular Biology Cambridge; F. Sherman, Rochester).

*Held at Aarhus University, Denmark on 9-21. July. Except where specified this article describes communications presented at the meeting.

More recently it has become possible to search for suppressor mutations in higher organisms by combining developments in the genetics of these organisms with advances in molecular biology that enable the precise nature of many structural gene mutations to be determined. In this way it is possible to show whether unlinked mutations that revert a particular genetic phenotype represent informational suppression or merely the activation of silent genes. Only a limited number of selective systems exist for the isolation of variant clones from mammalian cell cultures and as a result mutant cell lines can only be obtained with mutations at a small number of genetic loci.

One locus that has been extensively studied is that coding for hypoxanthine-guanine phosphoribosyl-transferase (HGPRT). Clones partially or completely deficient in HGPRT can be isolated by selecting cells resistant to certain purine derivatives such as 8-azaguanine (8AG) or 6-thioguanine (6TG). Good evidence exists that mutations in the gene for HGPRT are the basis of the changes in cell phenotype (C. T. Caskey, University of Texas, Austin; M. R. Capecchi, University of Utah, Salt Lake City). From these variants it is possible to isolate revertants that have regained the enzyme activity by selecting cells growing in medium containing hypoxanthine, aminopterin and thymidine (HAT) although this procedure only selects revertants having more than 40% of normal HGPRT activity, as shown by autoradiographic analysis of ³H-hypoxanthine incorporation (J. E. Celis, Aarhus). As such it is not suitable for obtaining informational suppressors of mutations that inactivate the HGPRT gene, since these are unlikely to be able to restore the enzyme activity to such a high percentage of its normal level and not disrupt protein synthesis to a degree lethal to the cell. To be compatible with viability they will probably only restore the enzyme level to less than 20% of its normal value (Celis). The cell lines that can be shown to produce an altered HGPRT are only a small fraction of the variant clones resistant to 8AG and 6TG, and therefore this selection is not very efficient for isolating lines in which to study the process of mutation in animal cells or the structure-function relationship of HGPRT (Caskey).

Until recently it was impossible to verify the nature of the alteration in HGPRT defective clones, and so to classify these as missense, nonsense, frameshift or deletion mutants. However, this problem may soon be overcome. It has recently become possible

to screen clones for the presence of nonsense mutations in the HGPRT gene by introducing nonsense suppressing tRNAs from yeast into the variant cells. These tRNAs are isolated from specific nonsense-suppressing yeast strains and have been characterised by their ability to suppress nonsense codons in *in vitro* protein synthesising systems (R. Gesteland, University of Utah, Salt Lake City). They can be introduced into mammalian cells either by direct microinjection using glass capillaries (Graessman & Graessman *Proc. natn. Acad. Sci. U.S.A.* **73**, 366; 1976) or, on a much larger scale, by being trapped within red blood cells which are then fused to the recipient cells (Kaltoft *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2793; 1976). Preliminary experiments using the first procedure have shown, by autoradiographic measurement of ³H-hypoxanthine incorporation combined with videotape recording of which cells were injected, that yeast nonsense suppressors cause a temporary phenotypic reversion when introduced into certain HGPRT-deficient cell lines (Celis). The second procedure can also be used to bring about *in vitro* suppression of nonsense codons (Capecchi) but is less readily applicable to the screening of many clones for nonsense mutants. It should also be possible to introduce frameshift suppressor tRNAs, isolated from frameshift suppressing strains of yeast, into variant cells, but these tRNAs may be too specific in their action to provide a selective system for the majority of frameshift mutants.

By fusing two mammalian cell lines, each carrying the same type of mutation but in different structural genes, it will be possible to isolate a hybrid cell carrying two identical mutations. In such a cell there will be a very low rate of simultaneous spontaneous reversion to the wild-type phenotype in both genes and the stage will be set for isolating the first true suppressor mutations of mammalian cells. Informational suppressors in higher organisms are already being characterised in *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. In the latter, Waterston and Brenner (*Nature* **275**, 715; 1978) have described a suppressor of the paralysed paramyosin-deficient E1214 mutant that acts on a range of alleles and partially restores paramyosin in worms homozygous for the mutation. The paramyosin synthesised as a result of the action of this suppressor seems to be identical to the wild-type protein. In *Drosophila* the existence of informational suppression is well characterised genetically, although in no single specific instance is the biochemical mechanism established.

Some disorganised muscle mutants of the nematode *Caenorhabditis elegans* produce a shorter myosin heavy chain than normal (A. R. MacLeod *et al. J. molec. Biol.* **114**, 133; 1977). After cyanylation of the wild-type and mutant proteins with ¹⁴C-labelled cyanide, a reaction which breaks the myosins at their very infrequent cysteines and labels the N-termini of the fragments with ¹⁴C, and gel fractionation of the products of partial cleavage of each of these fragments with cyanogen bromide, it is possible to distinguish nonsense and deletion mutants. In a nonsense mutant only the largest labelled fragment from one of the cyanylation products will have a reduced mobility relative to the fragments from the wild-type myosin, whereas in most deletion mutants several fragments show a diminution in size corresponding to the size of the deletion (S. MacLeod, University of Cambridge). Besides providing a way of mapping deletion mutants within the coding region, this approach can also be used to detect suppression of nonsense mutations in the genes for the heavy chain of myosin. □



A hundred years ago

NEWS from Panama states that the volcano Cotopaxi is in a state of violent activity. Its crater is surrounded by ice and snow but the clouds of ashes and smoke rising from it can be seen even at Guayaquil on the shores of the Pacific.

A VIOLET coloured meteor, with a reddish train, was seen at Stanislas, Austria, on the evening of the 24th in the Great Bear and moving in a northerly direction. It is described as thrice the size of Jupiter.

... it is stated that carrier pigeons are "being turned to useful account" in a new direction in Germany, for Consul Ward writes to the Foreign Office "that the successful results attained by the establishment of communication between the two Eider lightships and the Port of Tønning, in Schleswig, by these means has led to its organisation" elsewhere. This mode of communication is, however, not new, as carrier pigeons were employed early in this country as a means of communication with the Bell Rock Lighthouse, as mentioned in my late father's "Account" of that work. The pigeons passed between the lighthouse and the shore—a distance of eleven miles in eleven minutes. The employment of these birds, however, was, I suppose, found to be more curious than convenient for they have long since ceased to be employed.

From *Nature* **19**, 7 November, 5, 17; 1878.

BOOK REVIEW SUPPLEMENT

THIS, the author tells us, is "not a work of science; it is a work *about* science". Its thesis is that, in the coming period, the social sciences will be transformed by contact with evolutionary biology, in the same way as, in the immediate past, biology has been transformed by contact with chemistry. Human nature, like the nature of ants, hunting dogs or primroses, is the product of evolution by natural selection. It does not vanish with the appearance of culture, but remains as a constraint on the kinds of ways in which people can behave, and the kinds of societies which they can construct. Knowing this, he argues, it is both possible and useful to discover the nature of these constraints.

Like Wilson's earlier *Sociobiology*, the book is bound to arouse controversy; indeed, it is intended to do so. I hope that criticism will be of things Wilson does say, and not of things he does not. As most criticism will come from previously entrenched positions, it may help if I briefly describe my own. On many points I agree with Wilson. I do see recent advances in evolutionary biology, particularly in the field of behaviour, both as important in themselves, and as bound to give rise to renewed speculation about the relevance of biology to sociology. I do not think it sensible to see man as the behaviourists see him, a *tabula rasa* on which culture can write what it will. I do regard our higher faculties, such as our ability to construct mathematical arguments or to have religious experiences, as requiring an explanation in terms of natural selection.

Granting all this, however, I was not persuaded by Wilson's *Sociobiology* that biologists have all that much to contribute, for two main reasons. First, even if there are "human universals" which act as constraints on our behaviour, their nature will be discovered by psychologists and anthropologists; we biologists can only make encouraging noises from the sidelines. Second, human societies change far too rapidly for the differences between them to be accounted for by genetic differences between their members (Wilson, I think, would accept this). Hence, as differences are what we are primarily interested in, there is little an evolutionary biologist can say.

What, then, of *On Human Nature*? First, and most important, I found it entertaining and stimulating. I still

have my doubts about how much progress can be made along these lines, but I would like to see it read both by students and professors of sociology. It would stimulate their imaginations to learn, on the one hand, that human societies represent only a sample of the animal societies that are possible, and on the other, that features of society they may have thought unique to man are found in other animals.

Wilson has defined his attitude to "genetic determination" more carefully in this book. Interestingly, he recommends Waddington's concept of canalisation as a paradigm for thinking

Constraints on human behaviour

John Maynard Smith

On Human Nature. By E. O. Wilson. Pp.246. (Harvard University Press: Cambridge, Massachusetts, and London, 1978.) \$12.50; £7.85

about human nature and nurture. When he speaks of a trait as innate, he does not mean that it will develop in a fixed way regardless of the environment. He means only that a trait will develop in a rather constant manner despite wide changes in the environment, while accepting that a sufficiently drastic change in upbringing (or perhaps a sufficiently early one) might produce a profound change in outcome. By saying that characteristics are innate, he means only that we acquire some characteristics very readily and others only with great difficulty.

How then are we to recognise what is innate? Wilson reminds us that, classically, geneticists do not ask whether characteristics are genetically determined, but whether differences are. The difference between blue eyes and brown is genetic, and between speaking English and French is not, but it is meaningless to ask whether eyes or languages are genetic or environmental, as both require both genes and an environment. This has seemed to me a serious difficulty with the search for innate human universals. Wilson's

answer is that we can look at the difference between all humans on the one hand, and all chimpanzees, say, on the other. Thus, if most human beings avoid committing incest this can be regarded as innate, because there are other animals (not, as it happens, chimpanzees) which regularly do commit incest. The fact that incest does occur in our species does not prove that there is no genetically determined tendency to avoid it, because Wilson is not using the term "genetically determined" to mean "certain", but only "likely in most environments".

This attitude can be further illustrated by his chapter on aggression. It opens "Are human beings innately aggressive? This is a favourite question of college seminars and cocktail party conversations, and one that raises emotion in political ideologues of all stripes. The answer to it is yes." Now the reason this opening raises emotion in political ideologues (including this one) is that it will be taken to mean that human beings are aggressive come what may, that war is inevitable, and that it is therefore a waste of time to work for peace. But it turns out that Wilson does not mean anything of the kind. By saying that we are innately aggressive, he means only that we have shown aggressive behaviour, including warfare, in most, but not all, of the cultural environments in which we have so far found ourselves. He emphasises that the word aggression has been used to describe many disparate patterns of behaviour. He ends the chapter with a discussion of how we might circumvent our tendency to be violent towards one another. Given that these are his views, I think that the opening words of the chapter are unfortunate. They will certainly provoke controversy, but it is likely to be of that singularly useless type which takes place between people who do not understand one another. Rather than speak of traits being innate, it would be more sensible to ask: What are the circumstances, genetic and environmental, in which a particular trait appears, and how can it be modified?

Wilson's attitude can perhaps best be illustrated by his treatment of human sex differences. All known human societies show some economic sex role differentiation, which shows considerable bias, in that some roles (for example, making tools) are performed in most societies by men, and others (for

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E. O. Wilson

example, making pots) by women, although the bias is never absolute. Both the role differentiation and the bias in it call for an explanation. There seem to be two possibilities. The first is that there is a physical difference between men and women (women bear and feed babies; men are stronger and swifter) and the roles adopted are a direct consequence of this. The second is that there are innate cognitive and temperamental differences between men and women predisposing them to these roles. As technology is reducing the importance of purely physical differences, it would be easier to abolish role differentiation, should we so wish, if the first hypothesis is true.

Wilson's position, in brief, is that there probably are slight cognitive and temperamental differences between the sexes, but that sexual exploitation has been exaggerated as society has become more complex. He makes it quite clear that whether such differences exist or not need not determine our aims in present-day society. We could choose to exaggerate existing differences, or to give equal opportunity (in which case, he believes, we would not achieve statistical equality in role performance), or we could deliberately aim at statistical equality. Thus, he makes it clear (as was not clear, at least to me, in *Sociobiology*) that he does not subscribe to the view that what evolution has produced is morally right.

In general, his attitude seems to me sensible. The physical differences between the sexes are clearly a product of natural selection, and provide a clue to the breeding system of our recent ancestors. As an evolutionary biologist, I would find it odd if there were not also psychological differences; if economic role differentiation is ancient, as it may well be, then selection favour-

ing a psychological difference is also ancient. Wilson offers two types of evidence for such innate differences. One, that girls exposed to masculinising hormone in the womb often behave in ways more typical of boys, is relevant and suggestive. The other, concerning behavioural differences between small children, is less persuasive; even if children are treated alike, they know their own sex, and will imitate adult behaviour.

This book will arouse controversy on many separate issues. Some will criticise it on the grounds that any emphasis on man's biological nature will be used to defend the *status quo*. I think such criticism mistaken. Women have been monstrously misused; it is natural that many of them are angry and frustrated. But the cause of women will not be helped by refusing to think dispassionately about the nature of sex differences.

There are many other issues. To what extent is a causal, scientific approach—whether sociobiological, Marxist, functional or whatever—appropriate to the analysis of human affairs? If the religious faculty, like the excretory, is a mere product of natural selection, where can we look for the basis of our moral beliefs? To me, the most interesting question is how far evolutionary biology can contribute to the human sciences. As I have explained, I am a doubter. But I have been wrong on this issue before. Ten years ago I regarded incest avoidance as an entirely cultural phenomenon; only a bigot could hold this view today. I hope Wilson's book will be read and debated; I hope, too, that during the debate each side will listen to what others are saying. □

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Rejoicing in multifarious nature

Richard Dawkins

Ever Since Darwin: Reflections in Natural History. By Stephen Jay Gould. Pp.285. (Burnett Books/André Deutsch: London; Norton: New York, 1978.) £5.50.

Photo: D. I. Crossley/AP
"THE author shows us what is revealed when we remove the blinkers which Darwin stripped from biology a century ago." Some overkill there, or an excitingly paradoxical striptease technique? The first essay in the book discusses Darwin's own coyness in not revealing his theory until 20 years after he thought of it, and I shall return to this. The quotation from the jacket blurb gives a false impression, for Stephen Gould's writing is elegant, erudite, witty, coherent and forceful. He is also, in my opinion, largely right. If there are elements of paradox and overkill in Dr Gould's intellectual position they are not to be found within these covers. *Ever Since Darwin* is a collection of essays which first appeared as a regular monthly column in a magazine called *Natural History*. Skillfully edited to flow in eight main sections, the 33 essays, of which I can mention only a sample, reinforce my feeling that scientific journalism is too important to be left to journalists, and encourage my hope that true scientists may be better at it than journalists anyway.

Gould's collection begins to bear comparison with P. B. Medawar's immortal *The Art of the Soluble* (Methuen, reprinting). And if his style does not quite make the reader chortle with delight and rush out to show somebody—anybody—the way Medawar's does, Gould is to be thanked for some memorable lines. No doubt puritan killjoys of Science for 'The People' will denounce the vivid and helpful anthropomorphism in "Reproduce like hell while you have the ephemeral resource, for it will not last long and some of your progeny must survive to find the next one" (p94). But on second thoughts they may be too busy plotting the abolition of slavery in ants (p265), or brooding over the deviationism of "Natural selection dictates that organisms act in their own self-interest . . . They 'struggle' continuously to increase the representation of their genes at the expense of their fellows.



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And that, for all its baldness, is all there is to it; we have discovered no higher principle in nature" (p261). My only recurrent complaint against the style concerns the journalistic adjectival phrase before names: "English biologist J. B. S. Haldane probably anticipated every good idea that evolutionary theorists will invent this century".

Ever since Darwin we have known why we exist and we have known at least how to set about explaining human nature. I agree that natural selection is "the most revolutionary notion in the history of biology" (p23) and I would toy with substituting 'science' for 'biology'. Childishly simple as it is, nobody thought of it until centuries after far more complicated ideas had become common currency, and it is still the subject of misunderstanding and even apathy among educated people. A microcosm of this historical enigma is the subject of Gould's first essay. Just as humankind waited centuries longer than our hindsight deems necessary before discovering natural selection, so Darwin delayed his own publication 20 years after he first thought of the theory in 1838. Gould's explanation is that Darwin was afraid of the psychological implications of his idea. He saw, what Wallace would never admit, that the human mind itself must be a material product of natural selection. Darwin, in fact, was a scientific materialist—a biological determinist. And we know how biological determinists can expect to be treated by their colleagues. No wonder he delayed publication.

In another essay Gould is encouraged by the genetic closeness of humans and chimpanzees to speculate that "inter-breeding may well be possible". I doubt it, but it is a pleasing thought and Gould surely exaggerates when he rates it "the most . . . ethically unacceptable scientific experiment I can imagine" (p54). For my ethics, far less acceptable experiments are conceivable, and actually done in animal physiology laboratories every day, and a chimp/human hybridisation would provide exactly the come-uppance that 'human dignity' needs. Gould is, in general, rather good at puncturing human speciesist vanity, and in particular he will have nothing to do with the myth that evolution represents progress toward man. This scepticism informs his valuable account of "Bushes and ladders in human evolution", and fires his scorn for attempts to rank human races as primitive or advanced. He returns to the attack on progress in the very different guise of the theory of orthogenesis, the idea that evolutionary trends have their own internal momentum which eventually drives lineages extinct. His telling of

the classic Irish Elk story gains freshness from his intimacy with the fossils of the Dublin Museum and gives the lie to the myth that palaeontology is dry and dull. His conclusion that the proverbially top-heavy antlers were important in social life is surely right, but he may underestimate the role of within-species competition in driving species extinct. Large antlers could directly have caused the extinction of the Irish Elk while, at the same time, right up to the moment of extinction, individuals with relatively large antlers were out-reproducing individuals with relatively small antlers. I would like to see Gould come to terms with the "orthoselective" impact of "arms races" both between and within species. He seems to approach this in his essays on the "Cambrian explosion".

Ooh-ah biology can be sold for its intrinsic fascination, but it is much better used to make a point. Gould tells us about a fly that eats its mother from the inside, about seventeen-year cicadas and 120-year bamboos, and about uncanny fish-decoying mussels. He employs the useful trick of first opening the reader's mind by boggling it, then filling it with an important biological principle. One principle I would like to have heard more on is that of the limitation of evolutionary perfection: "Orchids are Rube Goldberg machines; a perfect engineer would certainly have come up with something better" (p91; Rube Goldberg is the American Heath Robinson). My own favourite example, inherited from an undergraduate tutor, is the recurrent laryngeal nerve. It starts in the head, goes down into the chest, loops round the aorta, then goes straight back into the head again. In a giraffe this detour must be wasteful indeed. The human engineer who first designed the jet engine simply threw the old propeller engine out and started afresh. Imagine the contraption he would have produced if he had been constrained to 'evolve' his jet engine by changing a propeller engine one bit at a time, nut by nut and bolt by bolt! While on the problem of perfection, I think Gould exaggerates the relevance of 'neutral mutations'. Molecular geneticists are understandably interested in DNA changes as molecular events, and any that have no effect on protein function may reasonably be called neutral mutations. But to a student of whole organism biology these are less than neutral: they are not, in any interesting sense, mutations at all! If the molecular neutralists are right, their kind of neutral mutation will for ever be hidden from the field biologist and from natural selection. And if a field biologist actually sees variation in phenotypes, the question of whether

that variation could be selectively neutral cannot be settled in the biochemistry laboratory.

Several essays touch on aspects of the relationship of Darwinism to human society and politics. There is much humane good sense here and I agree with most of it. Although 'sociobiology' is inspiring excellent research, Gould is right that it has also lead to some second-rate bandwagoneering. "But was there ever dog that praised his fleas?" asked Yeats. Perhaps a dog may be held responsible for the fleas that he sheds, but only to a small extent. At the AAAS meeting in Washington this year, Gould and I witnessed an organised attack on his most distinguished Harvard colleague. Gould well deserved his ovation for the apt Lenin quote with which he disowned the rabble. But as he watched those pathetic fleas ineffectually hopping around the stage chanting, of all things, "genocide", did he wonder with a little itch of conscience on which dog they had been sucking?

The Epilogue is forward-looking, and whets our appetite for the Volume 2 which I earnestly hope will be forthcoming. One theme which I know Gould has already carried further in his *Natural History* column is his dislike for "the ultimate atomism" of regarding organisms as "temporary receptacles . . . no more than instruments that genes use to make more genes like themselves" (p269). In describing this as "metaphorical nonsense", Gould underestimates the sophistication of the idea, first cogently expressed in its modern form by George C. Williams (*Adaptation and Natural Selection*, Princeton University Press, 1966 pages 22-25 and 56-57). The dispute is largely semantic. Inclusive fitness is defined in such a way that to say "the individual works so as to maximise its inclusive fitness" is equivalent to saying "the genes work so as to maximise their survival". The two forms are each valuable for different purposes. Both contain an element of personification; it is dangerously easier to personify organisms than to personify genes. The gene selection idea is not naïvely atomistic, as it recognises that genes are selected for their capacity to interact productively with the other genes with which they are most likely to share 'receptacles'; this means the other genes of the gene pool, and the gene pool may therefore come to resemble a "homeostatically buffered system" tending to return to (one of) its evolutionarily stable state(s). Irrevocable determination by genes is no part of the idea, nor is anything remotely approaching a "one gene one trait" mapping from genotype to phenotype. In any case it has nothing

to do with "supreme confidence in universal adaptation" (p269), which is as likely to be found among devotees of "individual selection" or "species selection".

"I will rejoice in the multifariousness of nature and leave the chimera of certainty to politicians and preachers" (p271): a resounding conclusion to a stimulating book—the work of a free and imaginative scientific mind. The final, sad paradox is this. How can a mind capable of such rejoicing, open enough to contemplate the shifting splendour of three thousand million years, moved by the ancient poetry

written in the rocks, how can such a mind not be bored by the drivelling ephemera of juvenile pamphleteers and the cold preaching of spiteful old hardliners? No doubt they are right that science is not politically neutral. But if, to them, that is the most important thing about science, just think what they are missing! Stephen Gould is well qualified, and strategically placed, to strip away even those dark blinkers and dazzle those poor unpractised eyes.

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Evolutionary advantages of sex

Alan Robertson

Evolution of Sex. By John Maynard Smith, Pp.222. (Cambridge University Press: Cambridge and London, 1978.) Hardback £9.50; paperback £2.95.

"WHAT song the syrens sang or what name Achilles assumed when he hid himself among women, though puzzling questions, are not beyond all conjecture", and so it is with the evolution of sex. Maynard Smith takes up again points which were first discussed by Darwin and, often with tantalising brevity, in Fisher's classical book of 1930.

Maynard Smith himself added a most important point to the argument in 1971 that in sexual species there is an immediate selective advantage for a gene which would allow females to reproduce parthenogenetically. In a sexual population of fixed size, each individual has on the average two progeny in the next generation and each gene contributes on average one copy. Suppose a mutant occurs which, without reducing the numbers or the vigour of the progeny of a female allows her to reproduce parthenogenetically. She would still contribute two progeny but all their genes would come from her so that the new mutant would be represented on average by two copies in the next generation. The mutation would then spread rapidly through the population.

Thus, parthenogenesis passes the first test to be applied to any potential evolutionary change in a species—will the genes which favour it have in consequence a selective advantage. Such mutants are clearly possible. *Daphnia*, for instance, has adopted a strategy of

cyclical parthenogenesis, breeding parthenogenetically in summer and sexually as winter approaches. A strain of turkeys is known in which a proportion of unfertilised eggs hatch—not very usefully in this case because all such progeny are male.

But there are further tests. Will species which have adopted this strategy be able to compete against their parental sexual species if the environment suddenly changes (for which the genetic variation in the species at any time is important) or when the environment is slowly changing over thousands or millions of generations, so that the species depends for successful change on its ability to accumulate new useful mutations, and also to get rid of the harmful ones. These are properties of the species as a whole rather than of its component individuals, so that competition is between populations—'group selection'.

A new and 'successful' parthenogenetic species, having already passed the first test in its present environment will be less likely to pass the second two tests, as Muller showed. It will clearly be more genetically uniform at any time. The majority of new mutants will be harmful and the species will be less able to tolerate them than will a sexual species. This is Muller's famous "ratchet mechanism". He showed that asexual populations below a certain critical size, depend on the overall mutation rate to harmful genes and their average selective disadvantage, will decline in fitness, essentially because of continued chance of loss of the fittest genotype.

Muller also showed that a sexual population is very much more efficient in accumulating new advantageous mutants, at least in situations in which many such selective substitutions are occurring at the same time. In an asexual species, the only way that two advantageous mutants can be combined in the same clone is by mutation in that clone, whereas in a sexual species recombination is possible of mutations which originally occurred in

different individuals. Muller presents these two arguments separately but I suspect that, as Felsenstein has pointed out, they really are aspects of the same process. Thus, we have two ways in which group selection will in the long term favour sexual compared with parthenogenetic species.

Maynard Smith's approach is to present first the theoretical aspects of questions and second to examine the available evidence in both plants and animals. He concludes that, though group extinction must play an important part in maintaining sexual reproduction, there must also be some short term advantages for sex.

It may not have escaped the reader that all the long term advantages of sex come from the independent assortment of chromosomes and that they therefore apply equally well to the maintenance of recombination. There is good evidence that the amount of crossing over on a given region can be altered by selection so that the raw material for natural selection is there. Theory would suggest that in a large population in a uniform environment, natural selection would in the short term favour a reduction in crossing-over. This requires epistatic interactions affecting fitness between genes at loci fairly close together on the chromosome. The arguments about the long term effects are the same as I have indicated for sex itself—indeed as initially presented the arguments referred to recombination rather than to sex.

Are there any short term advantages for recombination? Two are presented. The first, due to Maynard Smith himself, is in terms of competition between sibs. He shows that, if the environment varies widely from year to year and is described by several different variables, an individual of a given genotype in whose progeny there is segregation has a higher chance of producing successful offspring than one which does not. This he confirms in a model with five environmental features and five loci, one locus controlling adaptation to one feature of the environment. He finds clear selective advantage for higher recombination. The model, however, turns out to be not very robust to changes in the assumptions.

There is an alternative explanation, presented in a way that I found very obscure, even though I am an author of what is said to be the key paper. After much struggle, I arrived at an understanding as follows. Muller showed that, in the long term, a sexual species is better at accumulating advantageous new mutations and getting rid of bad ones. With no recombination, two useful mutations which have occurred in different individuals cannot be combined. Looking at the whole population,

there is then a complete absence of gametes containing both. Felsenstein showed that, in one special case, Muller's argument could be extended to short term selection—gametes which had arisen by recombination would be fitter than those which had not.

Our paper dealt with selection in finite populations, and we showed that, if two advantageous linked mutants are under selection at the same time, they tend to block each other's progress, because, under the joint action of selection and random sampling, there is in such populations a shortage of gametes containing both mutations. Increased recombination then speeds up selection. But Muller says nothing about population size. On reflection, a critical part of his argument concerns the occurrence of new mutants as units—the total number of copies of any mutant in the population is certainly finite at critical stages. So perhaps our statement contains Muller's as a special case. Felsenstein was able to confirm by simulation that recombination would be selected for in finite populations in which new mutants, either advantageous or harmful, are occurring. I should say that I remain a little uneasy that such a ma-

jor problem, the evolutionary implications of recombination, turns out to depend on such a 'second-order' mechanism.

Using the same general approach, the final third of the book deals with a series of secondary but related problems, such as the evolution of hermaphroditism, the avoidance of inbreeding, the evolution of anisogamy and the sex-ratio, and finally, thrown in for good measure, a discussion of optimal mutation rates.

I found the book stimulating in that it took me down many paths that I am not used to treading. It contains a fair amount of mathematics, which is almost all of the 'nuts and bolts' kind. But I did find it difficult. There could of course be three reasons for this—the author, the nature of the material, and finally the reviewer. I must say that I was a little taken aback when, having completely failed to penetrate a paragraph in one of the papers referred to, the authors then proceeded; "but the argument cannot really be this simple." □

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Shaking off the magic

P. B. Medawar

Magic, Science and Civilization. By Jacob Bronowski Pp.104. (Columbia University Press: New York, 1978.) \$8.70.

BRONOWSKI believes that a radical and irreversible complex of changes in the character of civilised life took place between 1500 and 1700: that which followed the eruption (*sic*) of science into western civilisation. It is characteristic of the rather lazy and flabby character of this book that although he clearly does not mean it he refers to the complex of changes as a 'step' and likens it to a mutation. The "prime contemporary issue" is "the place of science in the total field of knowledge and that of human values as we have to reformulate them in this century".

Bronowski believes that round about 1600 (give or take a century) it became possible for the first time to propound a unitary theory of the world: "to see the world as a whole and from a single core or type of explanation". He mentions grave and well recognised objections to this view, without in my opinion, countering them.

Bronowski is rather good on magic, of which his definition is admirably simple: "It is the view that there is a logic of everyday life, but there is also a logic of another world. Science is distinguished from magical views by the fact that it refuses to acknowledge a division between two kinds of logic . . . There is only one logic; it works the same way in all forms of conduct and it is not carried out by any kind of formula . . .".

Alchemical notions had a profound influence on the development of chemistry and chemical technology and in comparatively recent years it has become clear from the research of Frances Yates, Paolo Rossi and Charles Webster that magical, supernatural and literally scriptural notions played a most important part in the thought of many of those whom we think of as pioneers and champions of modern science; in particular one thinks of Frances Yates's discernment of Rosicrucian and hermetic influences in, for example, the writing of Francis Bacon and his contemporaries here and abroad. Dr Joseph Glanvill, FRS, for example, was an ardent believer in witchcraft and at the same time a champion of the Royal Society and all it stood for. This division of mind must have been most unsettling: it is no wonder that a *Tempest* raged in Prospero's heart.

Modern science has shaken off magic but Bronowski is very clearly

aware of the degree to which a predilection for the magical and mysterious persists amongst laymen in the cult of various forms of contemporary irrationalism.

I thought Bronowski's book not nearly as original, profound or illuminating as one might have hoped from

a man of his learning and intelligence. It gives me the impression, too, of having been rather lazily written, as if it had all come too easily to him. □

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Power of the scientific metaphor

M. J. Morgan

Programs of the Brain. By J. Z. Young. Pp.325 (Oxford University Press: Oxford, 1978.) £5.95.

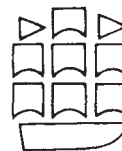
THE conceptual problems of biological development are perhaps clarified, but made no easier of solution, by the notion that the genes encode, not so much characters in the old-fashioned sense, but rather programs or sets of instructions for development. Until this metaphor became available, the only alternative to a crude preformationist kind of innatism was a mysterious force such as Driesch's "entelechy", which would allow the developing organism to survive the vicissitudes of the environment and the embryologist's knife. The idea of the program for development is novel because it allows a complete determinism to coexist with flexibility in development. To give just one example, a vertebrate limb bud on the left side of the body can grow into a mirror image of its normal form if transplanted on to the right side of the body, because its developmental program does not specify which limb it should be, but rather how it should grow in relation to surrounding tissue. An apparently marvellous example of flexibility turns out to depend on rather an economical program.

The analogies between embryology and the empiricist-nativist controversy in psychology are very striking, as J. Z. Young makes clear in this admirable book. There was a great need for someone equally versed in biology and psychology to write a book such as *Programs of the Brain*, and Professor Young has succeeded in getting the essential logical points across to the non-specialist reader. There will be no excuse, after reading this book, for confusing the proposition that innate factors control psychological development with the preformationist concept of 'innate ideas'. Equally, it is made clear that 'learning' cannot be invoked in complete opposition to nativism, as

learning itself is nothing but a program for acquiring certain kinds of knowledge. Stones, after all, learn nothing, and different animals learn quite different things. This reworking of the nativist-empiricist controversy has been commonplace in ethology for some year now, but it is useful to have it so clearly brought to the attention of the general reader.

Logical clarity alone, however, does not tell us how things work. And the unsatisfactory feature of this book, as J. Z. Young freely admits on several occasions, is that we really don't know how 'programs of the brain' work at all. The problem with a metaphor such as 'program' is that it gives an illusion of understanding, an illusion that gains powerful force from mere repetition. A previous generation of psychologists was excited by the telephone exchange model of the brain. The computer analogy is very probably an improvement, but as this book concludes with proper detachment, in the future "... perhaps a new form of laughter will also echo through the system, as the information comes up that in 1978 the best way the ancients had to describe these processes was to call them *Programs of the Brain*". One does therefore wonder what is to be gained by saying that there are programs for "loving and caring, fearing, hating and fighting, hearing, speaking and writing" (to mention but a few from the chapter headings). We are given very little clue as to how these programs work, and the mere verbal redescription smacks uncomfortably of the behaviourist's irritating habit of relabelling something like play as "ludic behaviour" and thinking that they had said something substantial.

One interesting hint J. Z. Young throws out is that brain programs for vision may depend crucially on topographical representation of the input. He suggests that our capacity for solving problems by visual imagery may depend on cortical maps of this sort. It would be interesting to have a further working out of this idea, which—understandably in a book of this scope—is not expressed in anything more than an allusive form. The idea seems to be that visual representation may consist at least in part of 'symbols', which in themselves have some features of the represented state of affairs,



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rather than being mere arbitrary 'signs'. This useful distinction between signs and symbols, which J. Z. Young makes rather clearly at one point, is unfortunately blurred later on when he speaks of colours as 'symbols'. The more usual view is that colours are arbitrary signs for different wavelengths of light. When J. Muller gave the first systematic expression to the doctrine of specific nerve energies in 1838 (not 1848 as stated in this book) he was expressing the idea, well known to Hobbes and other philosophers, that the quality of a sensation depends on the nerve that is stimulated, rather than on some property of the stimulating agent. Young and Helmholtz later extended this to different nerve fibres, proposing that perceived colour depends on the nerve channel stimulated. This seems to make colour a purely arbitrary sign. But J. Z. Young is perhaps insisting on something more profound in calling colour a symbol. There would be no need for an animal

to respond to a wavelength at all unless it were significant in some biological sense, and it could be that colour is a symbol of this significance, as the phenomenologists such as Merleau-Ponty have argued, instead of being an arbitrary sign of a wavelength.

As this book is written for the non-specialist it is worth noting with satisfaction that it has been produced by the Oxford University Press for only £5.95, complete with many excellent illustrations. This excuses certain oversights, such as a systematic disordering in the page references to the Bibliography, as if the latter had been added to at some stage without corresponding changes in the index. When *Programs of the Brain* goes into further editions, as it undoubtedly deserves, one hopes that this minor imperfection will be put right. □

M. J. Morgan is Professor and Head of the Department of Psychology at the University of Durham, UK.

Gloss on scientific practice

Stuart Sutherland

How We Know: An Exploration of the Scientific Process. By M. Goldstein and I. F. Goldstein. Pp.357. (Plenum: New York and London, 1978.) \$17.94.

It is a truism that the finished product of scientific endeavour—the successful theory—is very different from the halting steps by which it is usually reached. *How We Know* attempts to describe both the methods of discovery and the product: it is more successful at characterising the former than the latter.

Half the book is devoted to three illustrative case-histories—Snow's proof that cholera can be transmitted through drinking water, Rumford's experiments on heat, and the history of research into schizophrenia.

Snow is often credited with being the founder of epidemiology and although his work is well known, the story is worth retelling in somewhat more detail than usual. The final proof of his hypothesis was based on his discovery that one district in London was supplied by two separate water companies, one of which drew its water from a comparatively uncontaminated stretch of the Thames, the other from a region containing much of the

sewage of London. Snow was able to show that although the houses supplied by the two companies were quite randomly distributed within the district, the death rate from cholera was ten times as high in the houses supplied by one company as in those supplied by the other. This finding was the culmination of much patient work. Snow already knew, for example, that doctors, who always washed after attending a patient, rarely caught cholera, whereas those "women of the working class" who laid out the bodies and made "the occasion one of eating and drinking" frequently did. Having formed the hypothesis that cholera was transmitted by swallowing "the ejections and dejections of cholera patients", Snow persisted with it in the face of some evidence that seemed incompatible. Not everyone who drank heavily contaminated water became ill: the same argument is still used by confirmed smokers as a disproof of the fact that smoking causes lung cancer.

Both the account of Snow and that of Rumford gain in interest by being told largely in their own measured and elegant prose: if only today's scientists had the talent and leisure to write like their predecessors, how much more pleasurable the pages of *Nature* would be. Like Snow, Rumford persisted in clinging to a hypothesis: he believed in the kinetic theory of heat at a time when the caloric theory—that heat is a substance—was widely accepted. He showed that within the limits of his apparatus heat had no weight and that when cannon muzzles are bored an indefinite quantity of heat could be produced by friction; he also discovered molecular diffusion, attributing it to

the motion of molecules. But although his theory was correct, there were too many facts that at the time seemed more consistent with the caloric theory for it to be accepted: the rival theory gave a better account of such phenomena as latent heat and the transmission of heat by radiation across a vacuum. As Martin and Inge Goldstein note, the caloric theory, although wrong, itself led to many discoveries, including the laws governing heat capacity and the transmission of heat from one substance to another.

The authors' account of schizophrenia is perhaps the least interesting of their three case-histories: our knowledge of schizophrenia has been advanced by many different investigators and the story does not bear the imprint of a single mind in all its individuality. Moreover, as they acknowledge, they omit a great deal of the evidence that bears on the nature of schizophrenia so that some of the conclusions they record stand up rather better than is evident from their description. For example, they do not mention the World Health Organisation's massive study which established that both the prevalence of the illness (at least in its 'nuclear' form) and its symptoms show little variation across nine widely divergent cultures.

Although their case-histories make agreeable reading, the conclusions the authors draw from them are hardly new. Scientific discovery usually stems from the more or less systematic testing of hypotheses; luck may play a role; the investigator must be ready to take advantage of the chance observation that may prove significant; intuition plays a large part; the mechanisms underlying creativity are unconscious and ill understood; and stubborn persistence in a hypothesis in the face of apparently contradictory evidence can lead to major advances. They forebear to point out that persistence can also lead to disaster—any scientist knows of colleagues who spend dismal years following out an unprofitable hypothesis or approach: it is impossible either to know in advance whether persistence will be rewarded or to characterise the intuitions which enable the best scientists to judge when persistence is warranted and when it is not.

On the progress of science, the Goldsteins take a Kuhnian position, but like him they tend to overdramatise the nature of revolutionary changes in scientific paradigms. It is true that in some cases, such as Newtonian physics, the theory of evolution, or relativity theory, a single piece of work has made a great many seemingly disparate facts fit together and has at the same time produced a fundamentally new way of looking at things. But even in

such instances many of the ideas were in the air at the time, and in other cases revolutions in ways of thought have occurred more insidiously. Pavlov and Sherrington, for example, had the fundamental insight that it was possible to investigate the nervous system by examining its input and output and inferring the intervening mechanisms, but much of Pavlov's own theorising was wrong and despite the importance of his research, Sherrington's *magnum opus*, *The Integrative Action of the Nervous System* did not itself integrate many facts. Moreover, the Goldsteins place little emphasis on the importance of new techniques, the development of which often leads to fundamental new insights.

Their discussion of the finished product of science is inadequate. They point out that the Azande's beliefs about witchcraft are internally consistent and impossible to refute either by appeals to common sense or logic, and elsewhere they note the similarities but not the differences between scientific and artistic endeavour. They raise but do not answer the question "Is what we have called the scientific method . . . a better, surer road to truth and understanding than any other?" Partly through Kuhn's influence, and partly perhaps through a loss of confidence in our own civilisation, it has become fashionable to lump science together with other systems of thought and to claim that it has no unique claim to objective truth: this tendency has surely gone too far.

Mystical systems do not enable us to control or to predict accurately events in the outside world though some may help us to control ourselves; most such systems could be as tenaciously held as they are if the world were very different from the way it is; science is not just one amongst many possible and equally valid ways of viewing the world. It attempts to construct models which fit the facts with the utmost precision, and which combine generality with economy. Works of art operate upon us obliquely, are often ambiguous and leave so much unstated that different people can project into them different significances. Scientific theories, on the other hand, attempt to state unequivocally all their underlying assumptions, to remove all ambiguity, and to arrive at a system that can only be understood and manipulated in one way.

Despite its failure to come to terms with the nature of the products of science, *How We Know* is an interesting, if slightly elementary, gloss on scientific practice. □

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Dynamic equilibrium of science

John Ziman

Science as a Human Endeavour. By G. F. Kneller. Pp.333. (Columbia University Press: New York, 1978.) \$17.45.

SCIENCE used to seem simple. There were Laws of Nature to discover. There was a Scientific Method. Scientists were clever people, although aloof and uncultured. The marvelous new devices that benefited mankind came with dangerous new ideas that sapped the consolations of religion. Science was inhuman and incomprehensible; it was fortunately useful, even if it was unfortunately true.

To the more realistic studies of recent years, science has begun to seem more and more diverse, and more and more paradoxical. Its subject matter can vary from butterflies upon the wing to neutrinos trapped in a black hole. Methods of research range over techniques as diverse as electronic data-processing, opinion surveying, cinephotography and the playing of simulation games. An experiment may involve launching a space probe to Venus—or persuading a student of psychology that he is (perhaps?) near to electrocuting an innocent victim. A theory may be all algebra, or all words—and it may apply to the symmetry group of an elementary particle or to the elementary behaviour of a small human group. Scientists themselves vary from the speculatively divergent to the pedantically convergent, from sober realists to wild fantasists, from saints to sinners, from the fastest wits to the slowest bores, from high achievers to utter bunglers.

In the making of science there is the ever-present contrast between the solidity of established knowledge and the fluidity of conjectured novelty. In its applications, the incalculable benefit of a penicillin cannot be squared with the calculated horror of a napalm. Scientists belong to the most universal, transnational world community; at the same time, they are the most trusted servants of their warring governments. Scientific knowledge is as objective as we can make it; yet it is created out of the most subjective sweat of the individual human brow.

This diversity, out to the limits of conceptual, technical, psychological, political and moral contradiction, characterises science as a human activity. It cannot be cramped into narrower formulae, such as "All science is quantitative", or "All science derives from technology", or "All science is revolutionary" or "All science must serve the State". It would be a profound error to recoil from the evident complexity into yet another over-simplified stereotype.

With this, I think, Dr Kneller would agree. His excellent chapter on "The Scientist as a Person" emphasises the historical diversity of personalities and styles of thought. But he has not faced up to the necessity of finding the unifying factors in this vast endeavour. There is more coherence than he discloses, in the philosophical analysis of competing research programmes, the sociological analysis of norms and invisible colleges, or the economic analysis of science policy. He has read about, and adequately summarises, these various manifestations of the contemporary study of science, but fails to capture the leviathan beneath the surface. This is a graver defect in a thoughtful and lucidly written book than the many minor errors of fact that would irritate a well informed reader.

The heart of the scientific enterprise is the dynamic bond between individual and collective forces. Personal attributes of curiosity, intelligence, creativity and ambition are cleverly harnessed by communal restraints of organised criticism, institutionalised competition and the kicks and half-pence of social esteem. The "search for order in nature", Popperian falsification, Kuhnian paradigms, referees and peer review, graduate education, and many other diverse and apparently contradictory features of science, fall into place around this tense and quivering bond. This is the secret of the "Scientific Method".

What comes out of this is a body of knowledge with a few general characteristics. To achieve an adequate degree of consensus, science must concentrate on the material, demonstrable, publicly shared aspects of the world. The product of long, deep and highly imaginative study may look as 'unreal' as quantum theory; but this is no more mystical or unpractical than the mechanism of an earthquake or the anatomy of a louse. Ultimately a scientific theory can only commend itself by its predictive power—by an evident coherence between a conceptual scheme and the results of deliberate action.

Because it has had to stand up openly to expert criticism, established scien-

tific knowledge is reasonably free from wild fantasies and obvious inconsistencies. But the product of fallible human beings inevitably contains many minor mistakes, as well as grand misconceptions derived from the general opinions of the day. These deficiencies must be allowed for—yet they are incorrigible by reference to any principle that lies outside science. Scientific error can only be driven out by further science. Such progress may be much slower and more difficult than we would like—and in some cases may even prove illusory. Yet the process of self-correction is inherent in the method of science.

Scientific knowledge, being active rather than contemplative, is eminently applicable. Sophisticated technology may derive in large measure from science, and may provide the means and the motives for the investigation of particular questions. But neither the needs of practice, nor human aspiration, can determine the outcome in advance. Scientific knowledge is as objective and neutral as a frying pan or a sword: it is fit for such uses as those who possess it may desire, and for which it is appropriate, but neither can cook nor kill on its own account.

For all the philosophy and political sociology in the world, these maxims cannot be seriously controverted. But the scientific endeavour is questionable in several aspects that are scarcely mentioned by Dr Kneller. What is the potential scope of the scientific "method"? We feel sure that it cannot encompass ethical or aesthetic

issues. When allowance has been made for the reflexive feedback of consciousness, can science provide reliable knowledge about human behaviour in general? Is the objective of a 'science of society' essentially self-contradictory—a bootstrap exercise in which one always ends up flat on ones back? This question is important, because every unjustifiable claim to scientific expertise in social affairs is a terrible danger to humanity.

Another serious question is whether the social system of science can stand the strain that is put upon it by modern society. The scientists of our day still live and work according to the principles of the academic science of the past. But will the dynamic equilibrium between individual and collective forces still be possible in the industrialised and bureaucratised technical organisations of present-day research? The fundamental contradiction of contemporary science is between the technocratic impulse to turn science into a pliable instrument of policy and the pluralistic view that it must be allowed to go its own way as a fertile but erratic source of potentialities for understanding and action. There is a real risk that in our greed for technological golden eggs we shall try to put this wild goose into a battery and kill it with too much rich food. For three centuries, science has been a magnificent human endeavour; can it survive its own success? □

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Limits to scientific growth

F. S. Dainton

Scientific Progress: A Philosophical Essay on the Economics of Research in Natural Science. By N. Rescher. Pp.278. (Basil Blackwell: Oxford, 1978.) £15.

As an undergraduate reading chemistry I found in the College Library a remarkable book by R. G. Collingwood called *Speculum Mentis*. The clarity and cogency of the argument and prose style almost seduced me from chemistry to philosophy. From this temptation I was saved by reading Hishelwood's *Kinetics of Chemical Change* which convinced me that to be a scientific specialist need not exclude other concerns. Why should scientists

not have something to say about philosophy and philosophers something to say about science from which their auditors could profit? Now, 46 years later, I recall with pleasure the enlargement of my understanding gained from reading not only the works of past giants but also the works on science and philosophy of many near-contemporaries like Medawar, Popper and Polanyi. I return repeatedly to peruse them and always with profit. Alas, I am also reminded of the growing volume of mediocre work purporting to deal with the philosophy of science, and science policy. Much of these writings obfuscate rather than clarify; their prose is loaded with impenetrable, imprecise jargon—the hallmark of the institutionalisation and decay of an intellectual enquiry—and I put them down never to return to them.

This book does not fit easily into either of these categories. The author is prolix to a degree; printing costs hold no terror for him and if 11 words can be replaced by 4 he is unerringly generous. Thus, for "Figure X illustrates this" he writes "the case is rendered

graphic by the picture of figure x" and for "After phase one . . ." we read "once the first phase has been gotten over with . . .". More significantly the book is stuffed with lengthy quotations and with tedious tables and graphs telling the readers what most of them know, namely, that scientific activity, however measured (for example, by numbers of scientists, by their publications, by their costs, by growth of techniques, and so on) has grown phenomenally in the past two centuries.

All this verbiage obscures the message which is simple and could be encompassed in one-fifth the space with five times the impact on the reader. The proposition is that scientific progress is not limited either by any intrinsic finiteness or by human cognitive power, but that, although knowledge begets knowledge, which accounts for the near exponential progress of science in the early stages, scientific advances now and in the foreseeable future require even more effort and therefore the deployment of more human, material and correspondingly financial resources. Consequently acceleration gives way to deceleration of scientific advances and the smoothed (for the author acknowledges the episodic nature of discovery on the short time-scale) curve of scientific progress over centuries will appear sigmoid. This is not to say that there is an asymptote which is constant so much as having a slight positive slope.

Two features of this conclusion, which I can accept as probably correct though unprovable, are puzzling. First, to whom is the conclusion addressed and what use should be made of it? Governments, their agencies and large private and national corporations who provide the bulk of the money for scientific research already know that it becomes daily more expensive in real terms. This fact rather than the argument of this book is what sharpens their choices and affects their priorities. Will it help the scientists, stimulate their endeavours, quench their enthusiasm or send them off in new directions? I do not know but I doubt it.

What will the philosophers make of the book? I am not competent to express a view as to its philosophical significance though I would guess that it would have a value in slightly deepening their perceptions of the extrinsic factors which now restrain the rate of advance of science but which leave their own subjects virtually untrammelled. Nor will the economists or philosophers of science bat an eyelid for there is no news for them in this book. But it would make useful background material for school teachers, whether of history or of science, and

for undergraduates in the liberal arts and science colleges.

The second puzzling feature is that although the author refers to human will as influencing the pace of scientific progress he scarcely touches the more fundamental question as to what potentiates this will, though this is clear enough.

Science like art, music and literature is one of the ways in which man learns more of his own nature especially in relation to the external physical and biological world. The pursuit of science is part of the quest for his own identity. Additionally, scientific knowledge gives him power to use the forces of nature for his own ends and may even change some of the values by which he lives. What seems to me to be important here is whether Western man, whom science has enabled to meet his biophysical needs will opt in the post-industrial society to satisfy his psychosocial needs and what effect this will have on his will to advance science. Even if, as Daniel Bell has indicated, the optional post-industrial society is the learning society, will man want to know more of Nature or more of the

nature of Man?

Although I have described the main thesis it should be made clear that in developing it the author refers to other interesting matters. Thus, the importance of technology, even its necessity, for the advance of science is clearly delineated, although the almost inadvertent way in which science can provide the seed of much technology is scarcely developed. There are provoking juxtapositions such as the increasing cost of pollution and the diminishing cost of computers, attempts to provide a taxonomy of subjects and of technological speciation. But even giving credit for these virtues the book is not remotely worth £15 for which price one is entitled to expect the absence of elementary spelling errors such as *polution Wertheimer* (the author of one of 3 "magisterial" reports upon which "the book can be regarded as a philosophic—economic commentary") and *George Babbington Macaulay* whose second page reference in the index I could not find. □

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Universalistic view of science

Stuart Blume

The Reward System in British and American Science. By J. Gaston. Pp.204. (Wiley: New York and Chichester, UK, 1978.) £13.40.

THE most important criticism which can be levelled at Professor Gaston's highly polemical work is not that the arguments it contains are in error (though in some ways I believe them to be) but that it embodies a minimising and depressing answer to the question "what should the sociology of science be about?" No scientist, whether physicist or sociologist, wants his specialty to be defined so as to exclude the questions he finds most interesting. Let me explain, though necessarily at some length.

Some 20 years ago the distinguished American sociologist Robert Merton set out certain rules, or 'norms', of social behaviour to which he believed scientists tended to conform. These norms, a sort of moral code, were seen as uniquely congruent with the goal of science: that is, the advancement of knowledge. (For example, one such norm was 'universalism': the notion that any contribution to science must be judged solely on its scholarly merits, and independently of the nationality,

sex, institutional affiliation or age of its author.) Conformity was secured by rewarding 'proper' scientific achievement, both formally (with medals, invitations to serve on this or that) and informally (by citation of work): symbols that scientists came to want. Out of Merton's observations grew a sociological research programme, central to which was investigation of the reward system of science.

Either by personal interview or with a mailed questionnaire, data were collected from groups of scientists. Indicators of each individual's contribution to science on the one hand, and the 'rewarding' or 'recognition' received on the other, were correlated. The point was to see whether such extraneous factors as where the scientist had been trained, or where he worked, influenced the reception his work received, independently of its quality. Some of these studies (including that by Sinclair and myself of chemists in British universities) indeed found such "departures from universalism". (We found that an Oxbridge affiliation (a post at Oxford or Cambridge University) was an advantage in chemistry.)

After reviewing some of these studies, Gaston disputes the interpretation which the authors placed on their findings. His view is that systematic departure from universalistic behaviour is unproven. Moreover, in so far as there is any truth in the previous authors' claims, what is indicated is that the reward system works differently in the various disciplinary and national groups studied.

In the central part of the book (pp55–119) Gaston sets out to see if this is true. Information on a random sample of 600 academic scientists (divided equally between physics, chemistry and biology, and between Britain and the USA) was collected from published sources such as *Chemical Abstracts* and *American Men and Women of Science*. The two countries are seen as exemplifying contrasting styles of organising and funding research, and the three disciplines different levels of "codification" (=development). (For reasons of space I won't take issue with the crude reductionism underlying this view of the relationships between the sciences, nor indeed with the somewhat misleading picture of the British educational and science policy systems presented.) Analysis takes the form of many pages of correlations and regressions, involving such variables as each scientist's number of publications, years of research, 'honours' received, citations to his work, and so on. In brief, the conclusion is as follows. Factors such as sex or the prestige of the university of training or employment make little difference in science. Scientists are honoured, their work cited, almost wholly according to merit: the system is "universalistic". Not wholly so, however, and the differences between the disciplines turn out to be greater than those between the two countries.

Gaston's major conclusion, that science is largely universalistic, implies that science is unique in the modern world: unique not just epistemologically but sociologically too. If race, sex, social origins, place of work are irrelevant to the working of the scientific system, this is scarcely true of any other sphere of modern life. The claim that science is universalistic implies (though this is not examined) that scientists' commitments and loyalties, all the things that they are and that concern them when they leave the laboratory, are irrelevant to their systematic interactions with each other. In Britain a public school or Oxbridge education may be an advantage to the physician, the civil servant or the soldier—but not to the scientist. That may seem reasonable, but one can go on. Does it seem as reasonable to hold that to be invited to serve on a top-level advisory committee depends solely on scientific achievement, and not at all on politics or whether someone works in Massachusetts or Wyoming? Women are said to be at no disadvantage in science, and the fact that the sample of 600 included only 3.7% women is nothing to do with science. As this universalism is said to be almost independent of political and organisational contexts of science, it should be

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Richard J. Pankhurst

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hard to conceive of a country in which the honouring of a scientist's work could be influenced by any dissident views on political matters which he or she may hold.

It is a fallacy to believe that people's concerns, loyalties and commitments are so rigorously compartmentalised: that the fact of parenthood is irrelevant outside the home, or of political commitment outside organised politics. If these things are indeed irrelevant in science, then surely those who hold this to be so are by now obliged to prove how and why this is and remains so. The onus of proof must be shifted.

The divergence of view between Jerry Gaston and I derives in part from our different methodological preferences. What is to count as evidence? Should we correlate membership of the Royal Society, the US National Academy of Sciences or the Soviet Academy of Sciences with "number of papers published" — or should we try to probe how, and on what basis, these bodies actually elect members?

We differ also on what we want our subject to be about. On page 14 it is stated "Scientists cannot be totally motivated to conduct research by the level of monetary rewards". But are they totally unconcerned? Is the growth of trades unionism among scientists

wholly irrelevant (to sociologists)?

The depressing thing for me, then, is the implication which the book embodies as to what the sociology of science is to be about. If the subject is about the operation of the "reward system in science" — indeed central to the research tradition which Gaston so proudly espouses — and if this works virtually independently of socio-political environment, then what should sociologists of science do? There is little point in studying the way the system works in a traditional society like India or a culturally divided one like Canada. Yet interesting work of this kind is being done. There is little place in such a sociology for studies of the way in which intellectual and social changes in a discipline interact with each other over time. Yet here, too, fascinating work is being done by sociologists, historians and philosophers working together. A crucial test of any research programme must be the range and the interest of the problems for research which it generates. However convincing his statistical correlations, on this test Professor Gaston's work falls. □

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Lifeboat or country club?

Thomas H. Jukes

The Limits of Altruism: An Ecologist's View of Survival. By Garrett Hardin. Pp.154. (Indiana University Press: Bloomington, Indiana, and London, 1978.) \$10.

ALTRUISM is a fascinating and inexhaustible topic for writers. The betrayal of kindness by ingratitude is a recurrent and compelling theme in drama. Throughout human affairs there runs a curious and shifting equilibrium between selfishness and generosity, but, as Hardin notes (p79) starvation puts an end to altruism. Human actions divide into opposing categories of egoistic and altruistic.

For some years, Hardin has discussed problems of exhaustible resources, often in terms of analogies. One of the themes is that of a shared pasture that becomes irreversibly depleted by overloading with individually owned grazing animals. This is well illustrated by the current international depletion of oceanic fisheries. In *Living on a Lifeboat* (p142), Hardin says that "To avoid unconscious suicide, we are well-

advised to pit one metaphor against another". His lifeboat metaphor, which surfaces again in *The Limits of Altruism* (p62) says that it is foolish for the well-to-do nations, living in lifeboats, to go on welcoming members of the impoverished nations aboard. The lifeboat will sink with all hands.

However, I think that inhabitants of the impoverished countries are apt to counter with another metaphor. The prosperous nations remind them of the wealthy members of a country club who have closed the doors to outsiders because the golf course and the dining room might become crowded. Does anyone believe that we in the developed countries can keep the swimmers around us from climbing aboard the lifeboat—or from overturning it? Hardin evidently believes this. He says that we should "preserve enough of the economic and social barriers between groupings of humanity so that the cancer of collapse can be localised" (p84).

He states with emphasis that "Selection is not, by its nature, for the good of the species. The survival of the species is an almost accidental by-product of the survival of germ lines" (p107). However, it is obvious that germ lines cannot survive if the species becomes extinct, so therefore survival of the species cannot be an "almost accidental by-product".

In our species, says Hardin, selection occurred on a tribal basis. Altruism was practised within the tribe, and non-members of the tribe were exterminated (p112). Yet today (p127) "we continue to fling money desperately at poor countries half a world away, hoping for miracles" . . . "If we

desire a world in which altruism can persist, we must reject the ideal of One World . . ." (p132). Whether such a rejection is feasible is not clear. □

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Early Victorian science

David Knight

Science in Culture: The Early Victorian Period. By S. F. Cannon. Pp. 296. (Neale Watson: New York; Dawson: Folkestone, UK, 1978.) \$17.95; £12.50.

It was with a plea for history in depth, published in 1964 and revised as a chapter of this book, that Dr Cannon made a reputation; as befitted the journal *History of Science* in which it appeared, this was partly a review of recent work, partly a discourse on method, and partly an elucidation of an historical event. The various chapters of this book are all of rather the same kind, several others being revisions of articles; together they form a rather curious and loosely organised but very stimulating book, which poses more questions than it answers. Some chapters are very informal, and read like lively contributions to a seminar; thus, on one page "Thomas Kuhn" is introduced in one paragraph, and becomes successively "Thomas" and "Tom" in those that follow it. In sundry asides, we learn something about the author; for example, *apropos* of a discussion of professional success: "It is nicer to sit at the top of a new multi-million dollar building in a world-famous institution and let the seekers come to you. This is what I do". The founders of the British Association might perhaps have felt something like that, though they would have blushed to write it.

Dr Cannon's desirable office is at the Smithsonian, and one might expect that museums would occupy a prominent place in her discussion of early Victorian science; but they do not. In general, botany and zoology, and the world portrayed in D. E. Allen's *The Naturalist in Britain* (1976) get short shrift; also omitted is discussion of medicine, in which men of science (even physical science) were trained and which was undergoing professional reorganisation during Dr Cannon's period. Nor is chemistry seriously discussed, although it could have been, as Colin Russell *et al.* have shown in their *Chemists by Profession* (Open

University Press, 1977). Morris Berman, in his *Social Change and Scientific Organisation* (Heinemann, 1978) presents the Royal Institution as a temple wherein Day and then Faraday, apostles of applied science, preached to improving landowners; but applied science is not one of Dr Cannon's interests, and the reader will find little to remind him that Britain was in the throes of industrialisation, or that important educational centres were being founded in London and the provinces.

Dr Cannon is exposed, as anybody who advocates the ideal of history in depth must be, to anybody who cares to point to what she has left undone; but if the historian really had to take everything into account before he or she wrote anything, then the discipline would wither away. So it is within its limitations, as an account of what she calls the "Cambridge Network", that the book should be judged; and there it is illuminating. This is upper-crust history; her protagonists share an admiration for Broad Church doctrine and physical astronomy, and are professionals in the lofty sense that they pose the questions which the ordinary cultivators of science then try to answer. This view of professionalisation gives one a moderately testable version of Kuhn's idea of paradigms, and Dr Cannon's discussion, in general terms and applied to the founding of the British Association, is most interesting. John Herschel is particularly important in her study, and she makes out a good case for her remark that to agree with him "was considered a mark of good sense in early Victorian England".

For her, Herschel and his associates in the "network"—of which Faraday was a kind of honorary member—played a vital role in bringing to birth what we call 'physics' out of natural philosophy by Humboldtian observational science. She admits in a note that this is a partial view, and that steam engines had something to do with it too, but her part of the story is fascinating, and here as elsewhere hoary myths are elegantly shown up. Her suggestion of most general interest is that up to the 1850s in Britain there was a single view of truth, with science, religion, literature and history being complementary, and Newtonian astronomy the accepted model of demonstrated truth. She sees this golden age

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or era of good feeling collapsing in 1859 when Darwin published the *Origin of Species*, and not merely two but a whole series of cultures emerged, each with its own experts, or professionals, and tests of truth. This is interesting, and may be true; but if so, it would only show that events in England moved more slowly than they had in France or Germany, where the process had begun much earlier. One may also suspect that a focus on her Cambridge men—a group dedicated to the unity of knowledge—makes one attribute too much importance to them, and especially to Darwin, who appears here as the only star of any magnitude in the life sciences; Huxley comes in as his accompanist, rather than as the introducer of the revolutionary new physi-

ology from the Continent. In the decade before 1859, religious doubt had spread widely in Britain, and novels describing it had become a *genre*; men such as Frank Newman and J. A. Froude were prominent agnostics owing little or nothing to Darwin or his circle.

One may suspect then that some of Dr Cannon's generalisations apply not far beyond the limits of her "network"; but like the professionals she describes, she poses questions that will set others thinking for some time (and indeed have already done so) and she makes methodological remarks of great value to anybody interested in the history of science. □

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Foster's school

R. K. French

Michael Foster and the Cambridge School of Physiology: The Scientific Enterprise in Late Victorian Society. By G. L. Geison. Pp.401 (Princeton University Press; Princeton, New Jersey, 1978.) £18.40.

HERE is a biography of Foster and an account of the Cambridge School of Physiology. It is not, as the subtitle suggests, about *The Scientific Enterprise in Late Victorian Society* as it is limited to "one small group of English physiologists . . . between 1870 and 1900" (preface).

Although we are not far removed in time from late Victorian science, there is still a great deal we do not know about some of it, including physiology: Geison's book is a step in the right direction. Modern fashions in history range from history of ideas to sociological history, and historians, if not the readers of historical books, look for more than the old-fashioned Life and Times biography. But only rarely have we enough information to make the most of both of these approaches.

The format of this book is determined partly by the demands of social history, but the book itself is something of a pilot study in an area which, as Geison insists, is still very little known. The result is that these modern historical tools cannot be used effectively, and the text gives the impression of being somewhat over-written, a little bit 'stretched'. Another reason for this is the book's origin as a dissertation.

The copious text is subdivided by elaborate headings, which suggest a

somewhat laboured reworking of the material. The reader might even be well advised to read first the "Concluding Reflections" which so lucidly and tersely tie together the strands of the book that they are an indispensable guide to the detail and documentation of the earlier pages. These are naturally more exploratory than books written later in life. In his exploration Geison has uncovered a problem with Foster and has made valuable progress in seeing how it is answerable. Foster's reputation is that of a man with great influence on his subject and at the centre of its institutionalisation, yet the further the evidence and explanations for this are pursued the more elusive they become. Perhaps this problem can be historically generalised as the 'Boerhaave problem'. Boerhaave was one of the most renowned medical teachers of all time, yet the reasons for his influence are difficult to pin down. He made no major discovery and his teaching contained few innovations. His successful synthesis of current systems must be part of the answer, but the resultant bland and

conservative doctrine does not convince even the historian of ideas that it was the complete answer. Such a historian is compelled to use other techniques.

Geison's problematic Foster faded early as a research physiologist without having produced "a signal piece of research of the first order", and as the quotations prefixed to Geison's introduction show, neither contemporary or later historical opinion could in any concrete terms explain Foster's influence even by reference to his teaching. So, even though the problem calls for new historical techniques, the evidence is insufficient for their use. Geison's attempt to use them is dutiful but not always productive; at one point he is "ready to give up in despair" (p143) in attempting to solve the "mystery of Foster's achievement [which] can never be entirely dispelled" (p188). In attempting to dispel it Geison very usefully investigates Foster's work on the myogenic origin of the heartbeat, his characteristically English evolutionary view of physiology, which form a strong 'history-of-ideas' theme for the book, and his tactics to promote his 'school'—for example his publication of his student's papers. Much more hypothetical is Geison's brief and flirtatious parallel between on the one hand the politics of Bismarckian Germany, Czarist Russia, and the neurogenic 'autocratic' theory of heartbeat, and on the other, the opposing, innate, non-centralised 'democratic' myogenic theory, and Foster's role therein.

Readers of *Nature* will probably find these remarks too carping, and will certainly find the book itself a very welcome piece of work, carefully researched, dealing with an important topic and necessarily interesting to modern scientists and historians. □

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Sir Michael Foster

Courtesy Master & Fellows, Trinity College, Cambridge

Fragments of Chargaff

Jerry Donohue

Heracleitan Fire: Sketches from a Life Before Nature. By Erwin Chargaff. Pp.252. (Rockefeller University Press: New York, 1978.) \$13.

It seems appropriate to set the stage of this review with a quotation by the author, one which gives a bit of insight into the character of the character that will be dealt with:

"I cannot but find it remarkable that almost all the recognition my work received has come from Europe".

Why does he, full professor and department chairman at Columbia, awarded two Guggenheim fellowships, elected to the US Academy of Sciences, recipient of the National Medal of Science (and that's no *petite pomme de terre*) think this? Perusal of the *Citation Index* for the years 1965-68, to choose a few years at random, shows three or more columns of entries for all four years, from predominantly American journals—is not citation recognition? Then why, in 1970, did he submit an important essay, in German, to an obscure German monthly, and eventually publish it in not quite so obscure Swiss journal? This same essay was later translated into English, published in *Science* (172, 639; 1971), and in this form "became, perhaps, the most widely read of my articles."

This book was easier to read than to review. The first time I read it, I, well, detested it, and the further into it that I ploughed, the more I was distressed by the author's naïveté, self-pity, anti-Americanism, propensity to quote in foreign languages, inveterate name-dropping, recondite vocabulary, and much more. However, the second time around I found myself trapped into admiration of his observations on: Science (the field, not the weekly tabloid), scientists (some of whom, alas, are only alluded to and not named), Columbia University, molecular biology, current research conditions, DNA, and many more, as well as his piercing personal opinions on almost everything. But more of these later.

One especially valuable feature is the inclusion of a name index, with identifications, which runs to nine pages. However, it is unfortunate that not all of the names dropped in the text are indexed. Furthermore; is it really necessary to have such entries as:

"Carter, Jimmy—US president
Ford, Gerald R.—former US president
Hitler, Adolf—Austro-German politician
Michelangelo Buonarroti—Italian artist

'Twould have better to have pruned out the obvious, such as those above, plus a plethora of more, and to have included most of those omitted, such as Biedermeier, Consden, Doniger, Gordon—where and who are they now? Other names found in the text were also unindexed; should not these have been included? for example:

Khan, Genghis—Mongolian politician and explorer

Terrible, Ivan the—developer of Siberian real estate

God—Supreme Deity

Because of the fragmentary nature of this book, I decided to cast this review in the form of numerous excerpts, letting the author largely write his own review, so to speak.

Language, and use thereof

Chargaff has a decided tilt towards quoting titles, phrases, sentences, and long (sometimes very long) paragraphs in foreign languages. These are often, but not always accompanied by translations, some of which are unnecessary, for example, *Radetzky Marsch* = Radetzky March. (This may be the fault of his copy editor, that bane of authors who wish to write in their own style.) I noted passages in German (his favourite), French, Latin, Russian, Greek, Danish, Spanish, and Italian; Chinese is conspicuous by its absence, as are Finnish, Hungarian, and Romansch. His vocabulary in English is nothing less than esoterically florid. Consider the following words: aleatory, antinomy, avarnal, biopoiesis, bourdon, chiliast, epigonic, hieratic, maieutic, mephitic, phalansteries, polymathic (I stopped at one dozen). If these send you scurrying to your Webster, join me at the bottom of the class.

Whether he is fluent in all of the above tongues I do not know. He admonishes:

"Reading Ronsard [French poet] or Goethe [German writer] or Blake [British poet and artist] in translation amounts to listening to a transcription of the B Minor Mass [by Bach, German composer] for the ocarina."

He refers to K. Kraus,

"probably the wittiest writer in the German language": "He considered language as the mirror of the human soul, and its misuse as a forerunner of black and evil deeds. A grammatical haruspex, as it were he read the barbarous and bloody times to come in the entrails of the daily press."

Totally charming.

And who cannot be engulfed by such liquid prose as:

"I tell what I am told. Who is the speaker? If it is memory, then why does it sometimes whisper, sometimes shout, often chatter, and mostly remain in sullen silence? Ancient telephone numbers from my parents' apartment in Vienna are followed incoherently by the timid half-smile on a little girl's face, and I am eight. Busy ghosts with briefcases run through corridors that no longer lead anywhere. Blind mirrors

reflect frightened faces; a bright-red satanic mill engorges bodies and excretes packages of phosphates; gold teeth are sorted, numbered, and melted down; all this to the accompaniment of the sweet and labyrinthine music of the fourth act of *Nozze di Figaro*. It is all in pieces, but the broken edges cut, and there is blood everywhere."

But then we find this sentence, also referring to K. Kraus:

"The syntactic cataract of his periods, delivered in a specially pointed voice, was an ambush, hiding associations of a shockingly unexpected immediacy."

Now what on earth does that mean?

Also mysterious is his characterisation of the untranslatable (he says) German word *Greis* as

"so expressive in its diphthongal despair." (?)

On scientists

"A few years ago, I reviewed a scientific autobiography, a shallow best-seller. In my article [unfortunately not included in the bibliography, although at least one book review is included, so the identity of the 'shallow best-seller' can only be guessed at] I had something to say about this species of book, and this I should like to repeat here..."

We read that he finds these

"a most awkward literary genre," [that] "most scientific autobiographies that I have seen give me the impression of having been written for the remainder tables of the bookstores," [and that] "scientists write their life's history usually after they have retired from active life, in the solemn moment when they feel that they have not much else to say. This is what makes these books so sad to read: the eagerness has gone; the beaveriness remains..."

This book has neither. To be anti-anthropomorphic, Chargaff appears as a mongoose, warily circling the cobras of the scientific establishment, feinting, but ready to strike without warning at the numerous vulnerable points, and it all makes very interesting reading.

"Great scientists are particularly worth listening to when they speak about something of which they know very little; in their own specialty they are usually great and dull."

There are notable exceptions to this, of whom Linus Pauling is perhaps the most notable; Chargaff himself is another, as evidenced by this very readable book, the fact that you must have your Webster at hand notwithstanding.

"Most people I meet in my or other universities seem to be rejects from IBM. In fact you can talk with them only in triplicate. Slaves or prisoners of NIH or NSF, of Xerox or Beckman—they are really the narrowest, the dullest kinds of experts or specialists; they are essentially molecular podiatrists: people who know all about the fifteenth foot of the centipede."

Passages like this make me wish that there was some way for Chargaff to reach a larger audience than this book will, to get his messages across. Appearing on TV talk shows to plug the book, as the author of a 1968 scientific best-selling partial autobiography did, is not the answer.

"In general, there are few things as lugubrious as the professional travels of scientists. This has to do with the professionalisation of our lives and with the unstated, unacknowledged, and possibly even subconscious, cultural imperialism of America. Wherever one goes, the same form of pidgin is spoken, the same cocktails, the same indigestible food, the same centrifuges, gradient makers, the same graphs, all superimposable. After some time, all lectures form one confluent blob; all lecturers stumble through the same ten minutes of automatic recitation; all problems are chattered to pieces, and then coalesce again to the same meaningless phrase, something like 'structure and function.' The brutal clatter of a nonsensical machine engaged in the so-called dissemination of scientific information overwhelms all thought and imaginativeness. Diogenes with his lantern, looking for a human being, would be out of place in these assemblies."

"For instance, the National Academy of Sciences [of which Chargaff has been a member since 1965], which is, after all, only a sort of Chamber of Scientific Commerce into which some very funny characters have entered through various backdoors, is widely regarded as the true receptacle of wisdom."

It would have been very interesting if Chargaff had appended a list of these very funny characters, together with their backdoors.

Some nuggets on molecular biology

"One of the obnoxious dogmas to which it [molecular biology] has given rise—the so-called Central Dogma: DNA makes RNA; RNA makes proteins—is no longer valid . . . But the fact that dogmas could be handed down from the mountains [there are mountains in Cambridge?] shows that science had changed disastrously".

"The definition of molecular biology, given there [in the dialogue *Amphisbaena*, a writing of his not included in the bibliography], as 'the practice of biochemistry without a license' has become well known and much quoted."

"One particularly crude sentence graced the beginning of one of the lectures (the italics are mine) [at a small symposium on biological macromolecules in 1961]: 'The essence of the dogma, so acceptable to our time, can be briefly summarized by the following diagram: DNA→RNA→Protein.' (It had not been acceptable to me; but then, I am not of 'our time'.) . . . Actually, this particular dogma, on which free-thinkers and believers could agree so joyfully, has worn unusually poorly. Only a few years later, the peddlers flourished by hawking the reverse dogma".

"There are even some confused people who believe that what is now called 'molecular biology' makes up all the science of life."

On Columbia University

To an outsider, Chargaff's views on the institution where he was on the staff from 1935 until his retirement forty years later, are nothing less than astonishing. Thus:

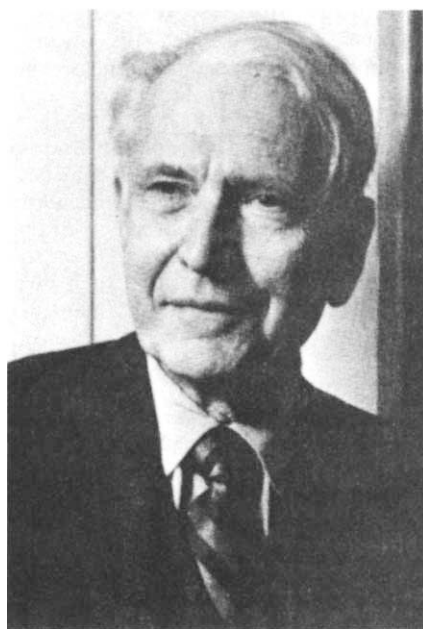
"Several [graduate students] already [in 1935] had reached distinction or were soon to do so, although the uni-

versity showed little recognition of this fact: an old Columbia habit".

And:

"Many years later, when I occupied the tabouret of biochemistry at Columbia, which in Clarke's time was still a real Chair, I wrote the firm [Eastern Kodak, where Professor Clarke, previous to becoming chairman at Columbia, had spent 14 years developing the imposing line of organic chemicals then and now sold by that firm], suggesting that they help us in setting up a professorship in Clarke's honor. The answer I received will remain a monument to corporate shabbiness".

Although this is less of a blast at Columbia than at Eastman Kodak, the least EK could have done was to have contributed towards a footstool, but that Chargaff chose not to publish that "monument" is regrettable.



Erwin Chargaff

And more:

"What made Columbia, during all my time, such a singularly disagreeable institution is difficult to explain".

Still more:

"Incidentally, I now have a simple method by which I can recognize future Columbia vice-presidents and deans, especially at the medical school: They misuse the adverb 'hopefully'; they 'address a problem'; they speak of 'input' as if they were dealing with a computer; they submit proposals as 'a package,' but if 'a package' is offered to them, they 'don't buy it'; they like to engage in a 'dialogue'—with several hundred people at the same time—but if you answer, they tell you that this is 'only semantics.' [Loud cheers]

Yet more:

" . . . I was dismayed to find that my pension amounted to less than 30 per cent of my last regular salary".

What kind of an *Elfenbeinturm* was this man living in? Did it never occur to him to ask someone? Granted that university bureaucracies are notoriously uninformative and mushy, it is, after some effort, possible to extract facts from them.

And finally:

"On November 20, 1975, the movers came. Certain things had to be left behind because they required individual attention, especially a cupboard full of my own old preparations. When we returned, we could not get into the laboratories. We were told that all locks had been changed at somebody's order . . . And since at Columbia one left hand never knows what the other does, it was quite fitting that less than six months after they had changed the locks on me at the Medical School, the University gave me an honorary doctorate".

The distinct impression is that the walls of Columbia are covered with *Rhus toxicodendron* instead of the *Parthenocissus quinquefolia* of her seven sister institutions.

On DNA and related matters

Chargaff describes the results of his early analyses:

"The regularities of the composition of deoxyribonucleic acids—some friendly people later called them the 'Chargaff rules'—are as follows: (a) the sum of the purines (adenine and guanine) [A + G] equals that of the pyrimidines (cytosine and thymine) [C + T]; (b) the molar ratio of adenine to thymine equals 1; (c) the molar ratio of guanine to cytosine equals 1."

He later refers to this paper as

"my ancient review article on nucleic acids, announcing the observation of the complementary relationships in DNA" (emphasis added).

First, if (b) A=T and (c) G=C, it immediately follows that A+G=T+C, so (a) is redundant. Next, let us look at the paper in question, the title of which is *Chemical Specificity of Nucleic Acids and Mechanism of Their Enzymatic Degradation*. The only molar ratios presented in the tables are A/G, T/C, and A/C, never the crucial A/T and G/C. Hidden away in the text is the sentence (emphases added):

"It is, however, noteworthy—whether this is more than accidental, cannot yet be said—that in all desoxypentose nucleic acids examined thus far the molar ratios of total purines to total pyrimidines, and also of adenine to thymine and of guanine to cytosine were not far from 1." [Calculations with data in other tables gives A/T values of 0.90 to 1.20, and G/C values of 0.94 to 1.33].

This hardly seems an announcement of complementarity.

This 1950 paper was based on lectures given in 1949. In 1951 there is another paper (*Fedn Proc.* 10, 654) in which, after noting that A/T and G/C are both "near 1," and (A+G)/(T+C) is "never far from 1," he remarks "whether these are merely accidental cannot yet be decided" (emphasis added).

In a 1952 paper, Chargaff *et al.* (*Biophys. biochem. Acta* 9, 402) give analytical ratios of, among others, G/C of 0.85, 0.90, and 0.93 for DNAs of several microorganisms, results hardly reinforcing the 'rules' with great vigor.

Who, according to Chargaff, made use of his rules? We now move to the

Cavendish Laboratory of Cambridge University which he visited in May of 1952, where John Kendrew asked him to speak with "two people who were trying to do something with nucleic acids". What happened:

"The first impression was indeed far from favorable; and it was not improved by the many farcical elements that enlivened the ensuing conversation, if that is the correct description of what was in parts a staccato harangue . . . The impression: one, thirty-five years old; the looks of a fading racing tout, something out of Hogarth ('The Rake's Progress'); Cruikshank, Daumier; an incessant falsetto, with occasional nuggets glittering in the turbid stream of prattle. The other, quite undeveloped at twenty-three, a grin, more sly than sheepish; saying little, nothing of consequence; a gawky young figure, so reminiscent of one of the apprentice cobblers out of Nestroy's *Lumpazivagabundus*. I recognized a variety act, with the two partners at that time showing excellent teamwork, although in later years helical duplicity diminished considerably. The repertory was, however, unexpected. So far as I could make out, they wanted, unencumbered by any knowledge of the chemistry involved, to fit DNA into a helix."

In the case of Crick, this unencumbrance apparently persisted for some time, for as late as 1966 (*J. molec. Biol.* 19, 548) we find him giving a drawing of a G-A pair featured by a non-bonded H . . . H distance between the bases of less than 1.7Å, a value impossibly small and ridiculously erroneous.

And then:

"I told them all I knew. If they had heard before about the pairing rules [*sic*], they concealed it. But as they did not seem to know much about anything, I was not unduly surprised . . . I believe that the double-stranded model of DNA came about as a consequence of our conversation."

Now, there are only a very few people who are in a position to know whether or not this belief of his is true. I am one of them. I categorically state that it simply is *not* true. Shortly afterward, when I came on the scene, at such times as they were thinking about DNA, they worried more about the density, the pitch of the helix; pairing, if it came up, was "like with like", not complementarity. They were not, in fact, even using the correct chemical structures of the bases. When the final model of DNA was discovered—more or less by accident—it wasn't Chargaff's rules that made the model, but the model that made the rules.

Finally:

"Later, when molecular prestidigitation ran wild, I was often asked by more or less well-meaning people why I had not discovered the celebrated model. My answer has always been that I was too dumb, but that, if Rosalind Franklin and I could have collaborated, we might have come up with something of the sort in one or two years."

Crick's own estimate of how long it

would have taken Franklin (by herself) to arrive at a correct structure is:

"Perhaps three weeks. Three months is likelier. I'd certainly say three months, but of course that's a guess" [see *Rosalind Franklin and DNA*, by Anne Sayre, Norton, New York, 1975, p214].

This crystal balling, whether by Crick or Chargaff, to estimate the time required for the successful solution of a research problem is of course totally fatuous. Chargaff's estimate of two years, however, may safely be expanded to "never", because as late as 1952 he was referring to "enolic hydroxyls" (*Biochim. biophys. Acta* 9, 399; 402). As everyone now knows, the Watson-Crick breakthrough is based on the fact that someone told them that there are *no* enolic hydroxyls in DNA, and had Chargaff used them an acceptable structure would never have been attained by him.

Some years previous to the "discovery of the century" Chargaff tells us that he "was in love with topology, and all forms of twisted rings filled the office. . ." and that "DNA was pictured as a Möbius strip." He then proceeds to give a wholly erroneous method for constructing such a strip. The love affair was obviously one-sided.

On science and research in our time

"I would say that most of the great scientists of the past could not have arisen, that, in fact, most sciences could not have been founded, if the present utility-drunk and goal-directed attitude had prevailed".

"Our kind of science has become so dependent on public support that nobody seems to be able to do any research without a handout. If their applications are turned down, even the youngest and most vigorous assistant professors stop all work and spend the rest of their miserable days writing more applications. This continual turning off and on of the financial faucets produces Pavlovian effects and a general neurasthenia that are bound to damage science irreversibly".

"One man or two may decide to study a certain beetle. Whether they do this because the animal is a pest or a biological delight is immaterial. If they find something of scientific interest, there will soon be ten others and more who will do the same. Once there are a hundred men studying the beetle, they will form a society and publish a journal. A society creates a profession, and a profession cannot be permitted to die. It is up to the nation to support it. If the nation can be persuaded, there will soon be a thousand members of the society for the study of the beetle. It is obvious that at this stage the beetle can no longer become extinct, for what would all these experts do who may well outnumber the beetle? Then a foundation will arise whose lay members—influential bankers, society ladies—will neither know nor care whether their function is to help with the eradication or the preservation of the beetles. They know one thing: they must support those who study the beetle. There may even be a Beetle Ball. But what if the beetle

disappears after all? The National Infantile Paralysis Foundation has avoided the worst by finally omitting its objective from its name. The American Cancer Foundation may find this more difficult".

Sic Transit

The penultimate section is entitled, *Liber Scriptus Proferetur*, and consists of a dialogue between the author and a "Voiceless Voice". It is a kind of *Höre mit Sinn, was ich dir sage*, and there is much said and much to listen to, mostly depressingly pessimistic, with the twilight glow gradually fading in the dim distance. Perchance the author is indeed a *Greis*.

Even though it may seem that I have some reservations, these, too, fade, with repeated reading of this book. Read it yourself, by all means. It is readable, enjoyable, enlightening, and at times hilarious without being funny. In the spectrum of scientific autobiographies which runs from the unbelievably dull to the delightfully enthralling, Chargaff's shines near the top. □

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Thirties science movement

Steven Rose

The Visible College: A Collective Biography of British Scientists and Socialists of the 1930s. By Gary Werskey. Pp. 368. (Allen Lane: London, 1978.) (Publication date: 16 November.) £10.

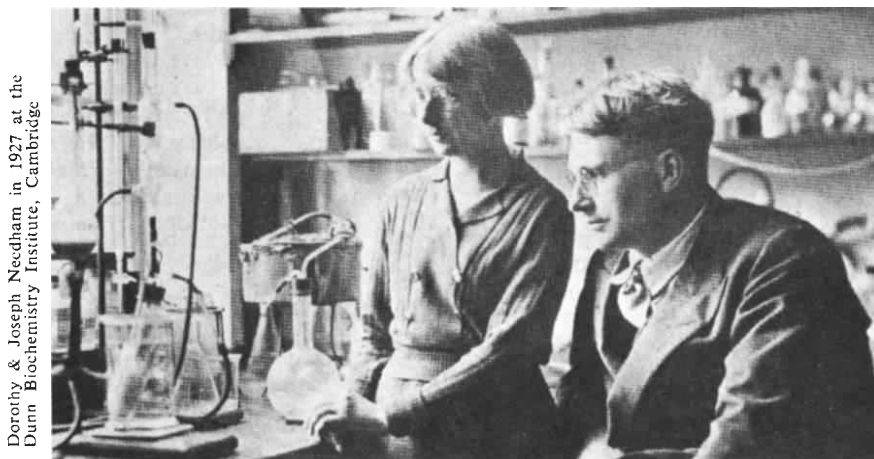
In the history of science as an institution, from the first tentative steps towards the organising of like-minded amateurs in the seventeenth century, to its present-day well-nigh total incorporation into the machinery of the state, the period between the 1914–18 and 1939–45 Wars occupies a position of particular interest and importance. For countries like Britain the Great War demonstrated the need for more decisive state and industrial intervention into the course of science and resulted in the establishment of forms of organisation—such as the Research Councils and Associations—which have remained with us through all the exponential growth phase of science spending which followed, until the present day. This expansion in the scale of scientific effort and rate of scientific advance (limited though it was by the depression of the 1930s), had a series of consequences.

An increase in the number of professional and qualified scientists and engineers and the resultant modification in their work style and class perspectives resulted in the first moves towards scientific unionisation with the National Union (later Association) of Scientific Workers, today merely a nostalgic memory in the maw of Mr Clive Jenkins' Association of Scientific, Technical and Managerial Staffs (ASTMS). In the superstructural world of the intellectuals science could no longer, as it had previously, be regarded with Swiftian or Peacockean disdain by the literati; what scientists said and thought about the world began to matter; *par excellence* this was the period of Snow's Two Cultures.

By the end of the 1939–45 war and the years that followed, the scientisation of everything had changed the terrain of that battle beyond recognition. As a result of these developments, particularly in the cataclysmic decade of the 1930s, science could no longer remain disengaged from politics at any level. What science was financed, and how, was a political question; what

science discovered, and how it proceeded, were political and ideological questions; what scientists said—about science and about politics—began to matter in a way that had never been the case before.

These changes excited, engaged, divided the 'scientific community'—by which one normally means that visible part of the profession which is university-based and regards it as its role to provide intellectual leadership. The divisions transcended academic debates and spilled out even from the pages of *Nature* into popular writings and political organisations. On the one hand there was a combination of mystically minded idealists like Jeans and Eddington, traditional conservatives with a dislike for the radical changes that were beginning to show in the scientific pro-



Dorothy & Joseph Needham in 1927 at the Dunn Biochemistry Institute, Cambridge

fession, like A. V. Hill, and proseletysers for a romantically conceived 'freedom' in the 'republic of science' like Baker and Polanyi. On the other, a formidable group of intellectuals with a range of talents within their disciplines and political and organisational skills that were to play their part in the total transformation of science in the decade that followed—Bernal, Haldane, Hogben, Levy, the Needhams, the Fremmins, the Piries, the Woosters, aided by a new species of science writers with access to the daily press like Crowther and Ritchie Calder.

The history of the science movement of this period is central to an understanding not merely of the political and cultural ferment of the 1930s, but to the appreciation of the intellectual and organisational origins both of British state science policy as it developed in the post-1945 period and of the new radical science movement which emerged in the 1960s. It is both surprising and slightly embarrassing that, although studies of the literary intellectuals of the 1930s have been so common, we should have needed to wait so long for the history which Gary Werskey has attempted in *The Visible College*.

Werskey's book has grown out of the thesis he embarked upon more than a decade ago now. His method is to analyse what we would today call the radical science movement, then referred to as the 'social relations' or 'social functions' of science movement, through a discussion of the activities and careers of five key figures; Bernal, Haldane, Hogben, Levy and Joseph Needham. He briefly traces the biography of each before the 1930s, trying to identify those factors in their development which led them eventually to socialism and for several, membership of the Communist Party. For Werskey's analysis the intellectual pivot around which the book revolves was the now famous International Congress of the History of Science in London in 1931, to which the Russians sent a highpowered delegation headed by the

major theoretician and Old Bolshevik Bukharin, which transformed the younger British scientists' understanding of the social processes of science by presenting them with a worked-out analysis of the historical and economic determinants of Newton's *Principia* as rooted in the needs of the emergent English bourgeoisie of the seventeenth century. The externalist analysis of the history of science was born.

In two central chapters Werskey discusses first the theoretical issues for the left scientists—essentially disparate attempts to define the relationships of science to dialectical materialism (particularly Haldane) and the social functions of science (Bernal)—and second, their practical work in the Association of Scientific Workers and, later, the Cambridge Scientists' Anti-War Movement. There is a brief account of the War and post-War years—disappointingly thin compared with the wealth of published autobiographical material available here—and a concluding chapter significantly entitled 'Coming in from the Cold'. Finally, an afterword very briefly summarises the heritage of the movement in the Janus-form of the almost total acceptance of state planning for science in

a capitalist Britain, and the rebirth of the radical science movement in the late 1960s around the issues of chemical and biological warfare and later the founding of the British Society for Social Responsibility in Science. Here, where my generation's autobiographies begin to interlock with those of the 1930s movement, Werskey leaves his story.

One should not underestimate the importance, or the difficulty, of the task Werskey sets himself. But I believe that in the last analysis he fails, and that the reasons for this failure are not uninteresting.

Firstly, the book is very uneven. It changes style and method as it progresses—the inevitable consequence perhaps of an overlong gestation period, and one during which the author's own political and intellectual

movement attempted would have avoided some of Werskey's problems. If he had based his analysis on the changing objective status of British science *vis-à-vis* British society he would not have been forced back into the sort of psychologising that wants to seek common features in the marginality of his key figures (to argue that Haldane's Scottish connections made one who was so solidly born into the British scientific and political aristocracy and Oxford society 'marginal' is surely stretching a point). His account of the Cambridge social scene of the 1930s is too uncomfortably reminiscent of Jim Watson's 1950s version in *The Double Helix* to carry total conviction. His essential unconcern for the scientific problems which Haldane, Bernal, Hogen, Levy and Needham set themselves also leaves him unable to see the im-

tions rather than changing social and economic circumstances. But also, one feels that Werskey has not been able to shake himself entirely free from a cultural and political background in which the American cold-war assumptions of his early training was followed by the essentially spontaneist radicalisation of the student movement of the 1960s. For him therefore, the Communist Party always appears as an external force motivated by Moscow, involved in Machiavellian schemes to control, neutralise or otherwise manipulate the misguided intellectuals. This is apparent in his choice of language; he speaks of Bernal and his "cohorts", a "left wing coterie" and so forth. Nor is his overall grasp of international communist theory and politics in the 1930s and 1940s absolutely sure, even on issues impinging directly on the science movement—for instance one should not argue that Stalin made a "sudden decision" in July 1948 to put T. D. Lysenko in charge of Soviet biology, especially after the re-analysis of the Lysenko affair in recent years by Lewontin and Levins, and by Lecourt.

What is of real interest is the essentially progressivist image of science that the old left had; science was inevitably in contradiction with capitalism and on the side of socialism. This was a view shared—and indeed Werskey hints at this—by the Communist Party with its belief in the 'scientific' nature of its version of Marxist theory. The alliance between socialist scientists and the Communist Party was thus inevitable. Indeed, it was a great deal more durable than that between non-scientific intellectuals in the 1930s and radical or Communist politics. The interesting point is the importance both sides attached to this alliance—for instance, natural scientists formed a significant segment of the Editorial Boards of the main theoretical journals of the Party and non-Party left in the 1930s and 1940s.

One of the most important differences between them and us is that the organised left no longer lays claims to such people; today its theoreticians are sociologists rather than scientists. Few of the leading cadres of the new radical science movement in Britain are active in scientific research in the same way as was the generation of the 1930s. The reasons for this change are surely connected with a completion of those tendencies in the development of the social functions of science which the 1930s heralded.

While Werskey's book remains the only history of the 1930s science movement, this analysis will, however, remain incomplete. □

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Hyman Levy broadcasting a science programme for the BBC in the 1930s



Haldane attempting to stir up a 'United Front' meeting in Trafalgar Square in 1937

positions *vis-à-vis* his protagonists and their goals have themselves undergone a considerable evolution—as he himself would, I am sure, be the first to agree. Thus, we move from a sort of psychologising biographical enquiry, seeking to root the socialism of Bernal and his colleagues in their early childhood, to an intellectual debate with the theoretical postulates of dialectical materialism as they were interpreted during the 1930s, to an account of a social movement and a puzzled attempt to come to terms with why left intellectuals in the 1930s saw the Communist Party as the proper and inevitable place to be.

The book is at its strongest when it discusses social movements like the Cambridge Scientists' Anti-War Movement. Elsewhere, it falters. It would be wrong to argue that only an insider could have written the history that Werskey has attempted, although it is true that a certain unease about the young American historian of science amongst the potential subjects of his enquiry has limited his access to some of the most interesting documentary material of the period, but nonetheless a consistent method of approach and a more basic sympathy with the intellectual as well as the social tasks that the Social Functions of Science

portance that they felt holistic methods opened up within their own subject areas, by contrast with either mechanical reductionism on the one hand or idealism of the Mach/Jeans/Eddington type on the other. Yet much of their best theoretical writing, and that of their colleagues and followers was addressed to just these questions. The analysis of the social functions of science was merely one aspect of the challenge that the left scientists presented; the issues and approaches that they opened in theoretical, population, developmental and human biology remain a profound and to date inadequately discussed theme (partly, it is true, due to the tendency of Haldane to mask subtle truths in outrageous *obiter dicta* and of Bernal to subordinate many of his theoretical concerns to those of the intellectual defence of the Soviet Union even where it was indefensible); nonetheless Werskey largely ignores this aspect of the group's work.

A further problem to Werskey is the role of the Communist Party and the relationship of his subjects to it. Again this problem partly derives from the technique of the tracing of five interlinked careers rather than the social movement and its objective base; he thus has to look for individual motiva-

Shades of Audubon

C. M. Perrins

Audubon: A Biography. By John Chancellor. Pp. 224. (Weidenfeld and Nicolson: London, 1978.) £6.95.

LAST year, an original set of Audubon's plates *The Birds of America* was sold at auction for a little over £200,000, the highest price ever paid for a copy of a printed colour-plate book. These extended-coffee-table works have captured the imagination of the world and a number of biographies have already been published. One therefore picks up another biography of Audubon with a little caution. The work under review, however, is a useful history of this man. It traces his family history and his early life in detail, following him through the period when he struggled to make a name for himself to his later life when he was already "a legend in his time."

Audubon was born in 1785, an illegitimate son of a French sea-going father. His mother is of uncertain history; she was called a "creole", but at that time this could have meant a number of things, from half-caste to a person whose family had lived in Latin America for several generations. The family background was one of the slave-owning communities of the southern states and Caribbean; indeed Audubon's father was for a time a slave trader himself. The father and two children returned to France at the time of the French Revolution and the young Audubon was brought up, largely by a stepmother, during these difficult times. He did not return to the New World until the age of 28. By this time he was already a dedicated naturalist and was drawing birds. Once in the States, he devoted his time to hunting, shooting and drawing to the extent that his business ventures failed with dismal reliability. He tried to run a number of merchant stores, moving westwards to Kentucky.

Always he hunted, painted and drew. In the early years he did not do so with any clear plan in mind for publication. However, such a thought may have been sown at a meeting with Alexander Wilson in 1810. Wilson, the first man to publish a book of coloured illustrations of American birds, approached Audubon to seek a subscription for his work (to be published as a serial). Nevertheless, another decade and a half were to pass before Audubon tried seriously

to follow suit. During this time his business ventures continued to fail and he and his family were often destitute. Although Audubon sometimes made ends meet by drawing and painting portraits, he never made much money from his natural history work. His wife, Lucy, whose marriage settlement he had long since spent, was often the mainstay of the family, earning a living as a teacher while her husband disappeared into the bush on hunting trips.

Things changed at last in 1826 when Audubon arrived in Liverpool and started to display his paintings in earnest. He met many enthusiastic subscribers for his proposed work and, after finding a publisher who was capable of doing the difficult work of preparing the plates (an Edinburgh firm failed in the early stages and most of the work was engraved by the London firm of Havell) things never really looked back. True, from time to time the number of subscribers dropped (at one time 50 of the 180 subscribers failed to renew their support) because Audubon was wandering in search of further birds and not trying to maintain or increase the number of subscribers; but he was never really short of funds again. By 1831, at the age of 46, Audubon returned to the United States a celebrity. In all *The Birds of America* comprised 87 parts, each of 5 plates—435 plates figuring 489 species. The first part appeared in 1827, the final one in 1838.

Audubon continued to visit England where he had a son overseeing the production of his work at Havell's. He continued to undertake expeditions searching for more species and he started new projects, including the



Painting of Audubon by his son



Barn Owls

Plate CLXXI of *Birds of America*

production of an octavo edition of his *The Birds of America* (1840–44), his journals (*Ornithological Biography*, 1831–39), and *The Viviparous Quadrupeds of North America* (1845–48). Many of these were undertaken with assistance of others. The text for his main work and for his journals was heavily re-written by the Scotsman William MacGillivray, himself author of an important work *A History of British Birds*; the text for *Quadrupeds* was written by the Rev. John Bachman, a long-time friend. Audubon died in 1851 before the completion of a new version of this work.

All this has been said a number of times in the numerous biographies. However, the work reviewed here does perhaps stand apart from some of the previous works which have tended to idolise Audubon. John Chancellor is more inclined to be openly critical of parts of Audubon's life. The artist made many enemies during his life and not without cause. He must at times have been an exceedingly difficult person to put up with. He was indolent and unfaithful to his trading partners. He treated his family badly. Even when he was becoming established as an artist he had little loyalty to many of his aides. The backgrounds for his artwork were usually done by other artists; they seldom got much credit and sometimes their names were deleted from the plates. His journals are contradictory and, to put it as politely as possible, Audubon tended to over-embellish the happenings on his journeys. He tried to put himself across as a colourful woodsman. Although it is not really fair to criticise Audubon for lack of conservation interests, as the idea of conservation barely existed at that time, he was wildly trigger-happy. A place where he shot fewer than 100 birds in the course of a day was rated "very poor for birds". It is a little wry

that a man who shot anything and everything should have given his name to the greatest conservation organisation of our time. On the credit side, his field observations were accurate compared with other contemporary works.

I would have preferred the author to say rather more about the artwork itself. As with the other aspects of Audubon's life, it has both admirers and detractors. Again, it is necessary to set the artwork in its time. There was very little else and what there was was stilted and un-natural, a point that Audubon was quick to stress. Not that, to the reviewer, many of Audubon's own paintings are of great merit in this respect. Many of the birds are depicted in grotesque attitudes which they could never achieve in life; this is especially true of the larger birds whose necks he bent into odd shapes to get them on the page. Perhaps the single most effective aspect of the artist's work was to draw the birds on so grand a scale, the

enormous elephant-folio size contributes to the impact of the plates.

The work reviewed here is profusely illustrated. Many of the illustrations are not drawings of Audubon's but works by other artists depicting, for example, places which he visited. Sadly many, presumably for reasons of cost, are monochrome not colour. In fact there are only nine colour reproductions of Audubon's bird work and two of his mammals. The reviewer would have welcomed slightly more detailed documentation and a larger bibliography. Nonetheless, the book makes interesting reading and will be appreciated by many who admire the artist's work and wish to learn more about him. The author has produced a thoroughly readable and attractively produced book. □

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Anecdotal history of Kew Gardens

William T. Stearn

In for a Penny. A Prospect of Kew Gardens: Their Flora, Fauna and Falballas. By Wilfrid Blunt. Pp. 218 (Hamish Hamilton and Tryon Gallery: London, 1978). £7.95.

WILFRID BLUNT is a master of anecdotal history and biography, as his works on such diverse characters as Sidney Cockerell, King Ludwig of Bavaria, Carl Linnaeus, Felix Mendelssohn, John Christie and G. F. Watts testify. In the Royal Botanic Gardens, Kew, he has found yet another subject for his witty light-hearted versatile scholarship and an approach which he correctly and disarmingly describes as "personal, capricious, irreverent" and "perhaps even prejudiced, for I have always had a kind of love-hate relationship with the botanists". His book is an entertaining combination of guide-book and history, well written and profusely illustrated.

As a botanic garden, Kew, although the mother of gardens in many parts of the world, seems young by comparison with the botanic gardens of Oxford (founded in 1621) and Chelsea (founded in 1673) and even more so with those of Pisa (founded in 1543) and Padua (founded in 1545). All these had originally a medicinal and teaching intent. Kew, which goes back as a

botanic garden no further than 1760, owed its existence less altruistically to royal pleasure, beginning with Queen Caroline, wife of George II, whose pet poet and tame hermit Stephen Duck (1705-56) has provided Blunt with material for an amusing marginally relevant chapter.

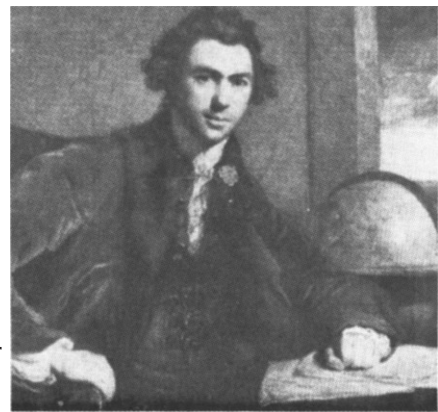
The horticultural zeal of Frederick, Prince of Wales, and his Princess, Augusta, determined the future of their royal Kew estate. Without them it could never have become the world's finest botanic gardens. In 1959 the Royal Botanic Gardens celebrated the engagement of a Scottish head gardener, William Aiton, as the founding of Augusta's Kew botanic garden, although Sir William Chambers, her architect, writing in 1763, had stated that the "Physic or Exotic Garden was not begun before the year 1760". Between 1757 and 1762 Chambers embellished the Kew park with a diversity of structures, of which the orangery and the pagoda stand as his most conspicuous monuments. Augusta's botanic garden occupied some nine acres of the present 300 acres forming the Royal Botanic Gardens. Blunt's first five chapters deal with this Hanoverian period and he joyfully incorporates its scandalous rumours about an affair between Augusta and her horticultural and botanical adviser, Lord Bute.

Augusta died in 1772. Under her son, George III, Sir Joseph Banks (1748-1820) took control of Kew, sent collectors far afield to enrich it with new plants and employed his botanist-librarians, Solander, Dryander and Robert Brown, to classify and name them and an artist of genius, Franz Bauer, to paint them. The result of

their endeavours was the *Hortus Kewensis* published as the work of W. Aiton and his son W. T. Aiton, "the names of native dunces being suffered to usurp the place belonging to those of the genius and talent of another land", according to Ker-Gawler in 1823. (The specimens on which this monumental work was based are in the British Museum (Natural History), not Kew.)

Banks gave the Kew garden an international reputation. Unfortunately, as Blunt says, "after the death of Banks in 1820, Kew entered upon the two darkest decades of its history. Indeed it sank so low that at one time what Augusta and Bute had so lovingly created, what Banks had so skilfully nurtured, seemed about to decline into kitchen gardens to provide cabbages for Kings" and might have ended as a built-up area.

The crisis came in 1838. Few of the many visitors to the garden and few of the research workers in the herbarium, libraries and laboratory know that, but for the efforts of a few influential and far-sighted men in the years 1838-42, they would have no Royal Botanic Gardens, Kew, today. The gardens were then under the control of the Department of the Land Steward, who was the Earl of Surrey, and the government, which would have been glad to be rid of them, set up a committee of enquiry for the purpose. It included the Earl of Surrey's head gardener but he was an ineffectual counterweight to Professor John Lindley, a man not only of great botanical knowledge and horticultural experience but also of forceful character, forthright speech and a vigorous lucid style, and Joseph Paxton, the Duke of Devonshire's head gardener, likewise a man of independent views. Lindley's report, advocating the conversion of the gardens into a public institution for promoting botanical science, was not what the government and young Queen Victoria wanted. Public indignation at the proposed destruction, however, led to the adoption of Lindley's views on the function and future of Kew, and in



Sir Joseph Banks

Painting by Thomas Deane, about 1773



Linnaean Society, London

1841 William Jackson Hooker came from Glasgow to be the director of the Royal Botanic Gardens, which under his guidance then developed into the botanical centre of the expanding British empire, as Lindley had hoped. I think Blunt does not give Lindley enough credit for his major contribution to the saving and development of Kew.

That development makes a varied story. Blunt tells it in fragments as he conducts his readers on a tour of the Marianne North Gallery, the Cambridge Cottage, the T-Range, the Herbarium and so on, though failing to do more than view from a distance the Jodrell Laboratory, which is a pity, as its forensic associations alone would have given him interesting material. Moreover, here C. F. Cross and E. T. Bevan did the early work leading to their discovery of viscose in 1892 and so to the rayon manufacturing industry. The celebrated plant anatomist and palaeobotanist, D. H. Scott, Honorary Keeper of the Jodrell Laboratory from 1892 to 1906, is likewise nowhere mentioned. Thus, although Blunt provides an excellent general view of Kew as a visitor sees it, together with a good historical background, the scientific work done there is largely and deliberately outside his chosen field. Indeed he describes his book as "an aperitif to the feast that Kew provides, not a meal to be consumed in the gardens."

Blunt asks "my friends the botanists to forgive a little gentle hadinage at their expense". They are, he says, "kind and delightful people"; he should therefore have asked more of their help over his shaky acquaintance with plant names. Thus, he attributes the coinage of the old Greek herb-gatherer's name ὄρχις (testicles) to Theophrastus, who simply used the common vernacular names of his times. "Why", he asks, "did they [the botanists] make *Orchis* feminine when the Greek word, and what it stands for, are masculine?". In fact modern botanists merely follow classical usage. The Ancients when using the word *orchis* not literally for humans but metaphorically for plants made it feminine in gender because most of their plant names were feminine and an association with a feminine noun such as ἔλαια (olive), *olea* or *herba* was implied. Thus Pliny wrote "mira-

bilis est orchis herba sive serapias, gemina radice testiculi simili." As regards the names of multigeneric orchid hybrid groups (see p177), the proposal to name these by attaching the termination -ara to the surname of a person was devised by E. A. Bowles about 1909, and put forward at the Brussels Botanical Congress of 1910, by which time such unwieldy condensed formulae as *X Brassocatlaelia* and *X Sophrolaelio cattleya* already existed. However, as Blunt says, "all of this has little or nothing to do with Kew".

It is unfortunate that he should repeat the old libel that work in the Kew Herbarium "adds to the misery of gardeners by constantly changing the scientific names of familiar plants". There is still room for a book covering the multifarious activities of Kew, which have included efforts to preserve unchanged the scientific names of plants. On p114 Kew and South Kensington are confused in a quotation from Beatrix Potter's diary; the entomologists Kirby and Waterhouse and the anatomist Sir William Flower were never members of "Kew's behind-

the-scenes staff"; they worked in the British Museum (Natural History).

The illustrations delightfully supplement the text; one portrays the director Sir William Thiselton-Dyer, "whose sense of humour was limited though that of his self-importance was not", as Blunt observes, parading in a constable's uniform with his garden constables. William Purdom, the rebellious Westmorland gardener who defied the autocratic Sir William, subsequently became a plant collector in China for Messrs Veitch and the Arnold Arboretum and travelled with Reginald Farrer. Indeed the history of Kew teems with names illustrious in botany and gardening, as well as some notorious, and Blunt has gathered anecdotes about them from multitudinous sources to produce a lively "personal, capricious, irreverent" book. □

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Crusade against malaria

L. J. Bruce-Chwatt

Mosquitos, Malaria and Man: A History of the Hostilities since 1880. By Gordon Harrison. Pp. 314. (John Murray: London, 1978.) £8.50.

THIS study by a "military and political historian and an environmentalist" (as stated in the author's preface) covers the period 1880-1976 of man's fight against malaria, the world's most prevalent tropical disease.

The book starts with the description of the discovery of the malaria parasite in Algeria by the French military surgeon Alphonse Laveran; it provides interesting and little known information on the background to this event and points out the difficulties that Laveran had in convincing the medical pundits (including William Osler) of the importance of his finding.

The next few chapters are devoted to Ronald Ross, undoubtedly the most colourful personality in the history of tropical medicine. Ross's "pilgrim's progress" from his first contact with Patrick Manson, through his fumbling, laborious and then inspired work in India, to the crowning glory of the Nobel Prize awarded for the discovery of the transmission of malaria by mosquitos, is told with much understanding and fluency.

Ross's bitter quarrel with the Italian investigators leads the author to an excellent appraisal of the major scientific contributions of Golgi, Grassi (Ross's "bête noire"), Dionisi, Bignami and Bastianelli. Here once again many interesting details, based on thorough research of original sources enliven the narrative. Robert Koch's little known and less than illustrious role at this stage of the history of malaria is discretely but skilfully reported. There follows the chapter on "three lives of the Plasmodium" which takes the reader through the explanation of the cycle of transmission of the human malaria parasites, including the cryptic tissue phase revealed in 1948 by Shortt and Garnham.

To introduce the concept of malaria control by the attack on the vector the author presents a sequence of events of the first quarter of this century, from Ronald Ross's first attempts at oiling



Ronald Ross

Photo: London School of Hygiene & Tropical Medicine

mosquito breeding places in Sierra Leone, through Sir William Macgregor's enlightened sanitation measures in Lagos, Malcolm Watson's brilliant successes in Malaya and then the controversial malaria control project of Indian Army's barracks at Mian Mir, near Lahore. (The discussion of the latter episode is particularly well researched and lucid).

A good popular account of the natural history of mosquitos precedes the description of the successful control of yellow fever and malaria in Panama, by General William Gorgas. This is also well told and provides many little known details of Gorgas's splendid effort in spite of the differences he had with Goethals, the commander of the Panama zone.

The next important phase of the fight against malaria opened in the early 1930s in Italy and the conflict between Angelo Celli's ideas imbued by his social reformer's spirit and Alberto Missiroli's growing faith in the new technology of control by elimination of adult *Anopheles* from human dwellings, is well brought out. The powerful stimulus of L. W. Hackett of the Rockefeller Foundation's research team in Italy is given a proper place in this part of the story.

Further chapters present the dramatic impact of malaria epidemics in Mauritius, Sri Lanka (Ceylon), Brasil and Egypt in the 1940s and the dawn of the technique of insecticidal spraying of houses as a promising method of control. The apparent failure of the Sardinian project of "species eradication", carried out between 1946 and 1951, was a prelude to the emerging concept of malaria eradication programmes which were set up at that time in Greece, Venezuela and elsewhere.

The last two chapters, entitled "India the model for the world" and "Relapse", cover the past 20 years of the global malaria eradication programme launched, coordinated and supported by the World Health Organisation since 1957. Here the author attempts to deal in a simplistic manner with all the complex problems that bedevilled this great and overambitious international endeavour. This part of the book fails to appreciate the many positive achievements of malaria eradication and seems to concentrate on pointing out the various deficiencies and errors—some true, others imaginary. However true certain remarks may be, the author's tendentious debunking of this major effort, with the air of someone who knows all the answers, is grossly unfair. His critical comments on the recent realistic appraisal by the WHO of the need to assess the costs of malaria control in relation to the means of the tropical

countries concerned, reveal his scant acquaintance with the socio-economic aspects of health problems of developing countries.

Oddly enough, there is no mention of the fact that the most stringent criticisms of the strategy of the global programme of malaria eradication came from the remarkable, self-auditing study of the World Health Organisation, namely the well known Annex 13 of the Director's General Report to the Twenty-Second World Health Assembly in 1969.

The achievements of the past 50 years of chemotherapy of malaria as an important weapon for prevention and treatment of this disease are omitted from this otherwise comprehensive study.

In summary, this is a patiently researched and very readable story of events that formed the milestones of the relatively recent knowledge of the transmission of the agent of malaria and its control by the attack on the vector. It is regrettable that the appraisal of all aspects of the attempted global malaria eradication is somewhat unbalanced, by concentrating on its failures.

There are several minor errors in the names of persons or titles of books (for example, Beauperthuy not Beauperty; Daniels and not Daniel; Macdonald not Mac Donald; Ross's romance was *The Spirit of the Storm* not *The Spirit in the Storm*). Some footnotes are perplexing: thus, the one on p40 says: "Ross knew that it was the female that sucked blood but military man, that he was, he persisted in calling her 'he'". A few instances of purple prose such as Ross's "adrenaline pump" or his "ecstasy of Ego" are jarring in the generally clear and pleasing style of a book that should nevertheless interest a wide circle of readers. The illustrations are well chosen: the copious notes are generally relevant and the list of references is valuable even though it shows some inexplicable lacunae.

The blurb on the cover mentions that "The extraordinary story of the crusade against malaria is told here for the first time". This is simply not so, because several excellent books on the subject were published during the not too distant past: Paul Russell's *Man's Mastery of Malaria*, of 1955, is still one of the best of them. Moreover the recent ups and downs of malaria eradication have been dealt with fully in published reports of the WHO and in numerous other articles. □

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Ecological sanity

Kenneth Mellanby

Reconciling Man with the Environment. By Eric Ashby. Pp.104. (Oxford University Press: Oxford and London; Stanford University Press: Stanford, California, 1978.) £4.25; \$7.95.

LORD ASHBY is a distinguished botanical research worker. He was Professor of Botany in Sydney and in Manchester. He was active in establishing the Society for Experimental Biology in the 1930s. He was also the first chairman of the Royal Commission on Environmental Pollution, set up by the British government in 1970 to "advise on matters, both national and international, concerning pollution of the environment; on the adequacy of research in this field; and the future possibilities of danger to the environment." The experience gained in this career has given him an ability to survey the environmental scene with unusual perspicacity.

In this book Lord Ashby is particularly concerned with the ways in which public opinion may stimulate governments to cope with hazards to the environment. He describes this as a "chain reaction", in which the interest of the public is first ignited by the media, then the hazards are assessed by scientists and economists, and, finally, political action is taken. This action is often based on a compromise between opposing interests, and may take into account both objective data and subjective values. In general the British pragmatic approach, with attention to concrete effects on man's surroundings rather than on rigid standards setting levels of emission, is shown to be most effective.

Successes and failures to protect the environment are illustrated by several examples. Lord Ashby describes graphically the struggle over Upper Teesdale in the north of England, where an area of unique botanical importance was the most convenient—and least expensive—site for a reservoir, needed for the industrial development of an area of potentially high unemployment. Although the reservoir was eventually built, the botanists and other conservationists who opposed its construction were given a good hearing and substantial modifications were made in the scheme to try to reduce its ecological impact. Funds for research in the area were also provided by the commercial interests most involved. Lord Ashby

infers that this demonstrates how, by the 1960s in Britain, public opinion had been educated. It is suggested that at an earlier date industrial necessity would have been encouraged to ride roughshod over the site, destroying the Teesdale Sandwort and the Spring Gentian.

Of more general topics, the examples of the great improvement of the quality of the air in British cities, and the moderate improvement of the state of the water in British rivers, both in the past quarter of a century, are quoted. These improvements have cost both industry and the public a considerable amount of money, something which might have not been tolerated fifty or a hundred years ago. In the nineteenth century many attempts to persuade Parliament to legislate were unsuccessful before the passing of the first Alkali Act (controlling little else but acid emission to the air) in 1867. The more drastic Clean Air Act of 1956 was generally welcomed, even if some of its most stringent provisions have not yet been adopted in many parts of the country.

In recent years there has been serious controversy about waste disposal, particularly of poisonous substances. Lord Ashby's Royal Commission realised this was a danger, and so reported strongly but soberly in its 1971 report. The government did nothing—the programme for parliamentary legislation was full up. The matter was then taken up, more shrilly, by various voluntary bodies of citizens concerned with the environment. They made the case more emotionally, but still relied for the main on a reasonable assessment of the dangers. Nothing happened—Parliament was still too busy. Then a large number of drums of cyanide were found, with their labels inefficiently removed, in an area of wasteland used as a children's playground. It was alleged that this "flytipping" occurred with the tacit encouragement of the driver's employing company. There was an immediate outcry, and within days Parliament passed a bill to prevent a recurrence.

However, I think that Lord Ashby may be overstating the contribution to the protection of the environment by the media, and of any widespread change in "public opinion". In the case of the toxic wastes, the drum of cyanide were a real and easily demonstrable danger, which dramatically highlighted the Royal Commission's warnings. Often it seems that there is only a public outcry about environmental problems after they have been solved by backroom experts using unglamorous means. This may be illustrated by changes in air pollution. It

is generally believed that the improvement in Britain occurred because of action following public outcry, when it was reported that the great London "smog" of 1952 killed off, prematurely, some 4,000 elderly, ailing and bronchitic citizens. The resulting Clean Air Act is thought to have been the main instrument of change. In fact London's air even by 1952 was far cleaner (or, perhaps, less filthy) than it had been in the nineteenth century, in the days of Dickens. The improvement was mainly because it became cheaper and easier for industry to use clean fuels like oil, gas and electricity, and because the competent and well informed Alkali Inspectorate was beavering away controlling industrial emissions years before the Act was passed in 1956. At the same time many householders had already stopped burning raw coal before regulations compelled them to do so. Legislation was useful, but only in accelerating a change already taking place.

The situation regarding pesticides is also interesting. Lord Ashby says that had not Rachel Carson published *Silent Spring* in 1962 "public apathy about the hazards of pesticides might have persisted a decade longer". This is quite true, but is hardly relevant to the way in which pesticides were controlled, at least in Britain. Here naturalists and ornithologists in particular had been concerned with the deaths of thousands of seed-eating birds each spring from about 1955 onwards. The situation had been investigated by them and by scientists in government laboratories, and seed dressings of aldrin and dieldrin on spring-sown wheat implicated. Some time before *Silent Spring* was published the voluntary ban on these seed dressings on spring wheat was operated, with the result that the bird deaths were greatly reduced. Even before that the successful Pesticides Safety Precautions Scheme was set up with the result that no new chemical causing comparable ecological effects on non-pest species has been introduced. It is possible to argue that the wide public reaction to pesticides, sparked off by Rachel Carson, has generally been counter-productive. Before the book appeared, informed public opinion had taken effective action. After the book aroused uninformed opinion, many, particularly in developing countries, have been denied the use of safe pesticides and so the incidence of, and death toll from, diseases like malaria has greatly increased.

However, I only disagree with Lord Ashby in the way we define "public opinion". Effective environmental control has required the support of the informed public, but not of the masses

in general. I realise that this view will today be castigated as 'elitist' but it is nevertheless true. Where mass opinion has been inflamed, overreaction has often occurred, unsubstantiated statements have been put forward, and eventually there has been a 'backlash'. This is being experienced in many Western countries today, where the majority of the population are becoming bored with the environment. The uninformed and over-reacting 'environmentalists', preaching doom and gloom, are becoming increasingly discredited.

Lord Ashby is certainly too well informed to have any truck with the doom and gloom brigade. He considers that man has been, to some extent, reconciled to his environment, and that this is a "qualified success story". He does not conceal the fact that real damage occurs, and he indicates how this damage may be mitigated. But he concludes his book by pointing out

other, real reasons for concern. These are man's prodigal use of non-renewable resources and the difficulty those people with different ideologies have of living together in harmony. The final, and greatest danger, is thus political. The only hope is that mankind will learn from the qualified success in the environmental field. Here the cumulative effects of wise decisions (what Lord Ashby calls "wise medium-term microdecisions, each clarifying the shape of the decision that needs to follow") has produced a promising result. We can only hope that the problems of diminishing resources and political incompatibility will be solved in the same unspectacular way, by the efforts of the responsible minority, not be a tumult promoted by the media. □

Kenneth Mellanby is editor of the international journal Environmental Pollution.

Politics of atomic energy

L. Rotherham

Nuclear Power and the Energy Crisis: Politics of the Atomic Industry. By Duncan Burn. Pp. 352. (Macmillan: London, 1978.). £12.

In his preface, Duncan Burn suggests that reviewers will be able to point out that some things are left out from this book but that the omissions will not affect the argument presented. There is a selected bibliography which includes about 100 items, and references in the text must number 700–800. Although the same reference may appear several times, no one would wish to say that the author has not carried out his work thoroughly. Some references unfortunately are useless: for example, taking only chapter 10, we find ref. 188/40—"I am following in this notes of discussions I had . . ." or 188/53—"This view was expressed to me early in 1964 by a member of the CEBG"—why not say which member, so that it can be authenticated?; or 191/109—"I had discussions at the time".

One would not wish to question the authenticity of the author's statements but he might have heard what he wanted to hear and we cannot say. Where he refers to published work, his excerpts seem to be exactly correct but his comments and his selection seem to be aimed to sustain his own opinions, and in the light of history that hardly seems necessary.

If this justification is the first theme of the book, a second is that the assessment of tenders for the Dungeness B power station was mishandled by the Central Electricity Generating Board (CEGB) and the Atomic Energy Authority (AEA), mainly due to the overwhelming influence of the AEA; and finally, it is suggested that the Government shares in the blame by interference in technical decisions where it lacks competence. These three themes are linked, of course.

The tenders for Dungeness B were received in 1965 and at the time virtually all British experience was in gas-cooled nuclear reactors, whereas in the United States nearly all experience was in water cooled reactors (LWRs) and in both cases the early experience was taken from military applications. In Britain, Calder Hall (a gas-cooled station) had been operating well since 1957 and rather larger stations had been commissioned for the CEGB in 1962 and 1963. These stations are still operating. Capital costs of the CEGB stations were higher than had been assumed but, of course, they are never lower and a subsequent de-rating of some of the stations amounted effectively to a further increase in capital costs. These facts are quoted in the book although they were not all known in 1965. Good influences such as the improvement in fuel element life are not quoted. It will be interesting at a later date to make a retrospective study of the cost of generating from these nuclear stations to compare with life-time figures from LWRs and with fossil fired stations. It is too early to say that the full truth has been revealed and that all is disaster.

In America, progress was through a

series of subsidised demonstration reactors but this changed dramatically in 1963 when the Oyster Creek LWR power station was ordered. The quoted price was so low that it was regarded as being either heavily subsidised or a lost leader intended to sweep the market. Chapter 4 deals with the quick increase in costs between 1963 and 1966 but Oyster Creek did establish the LWR as probably the lowest cost system and it was accepted throughout the world, except in Britain, Canada and Russia.

In 1965, the advanced gas-cooled nuclear reactor (AGR) was expected to have higher initial costs than the LWR but lower operating costs as it was designed to produce steam at higher temperatures and pressures than the LWR and was matched to the large turbogenerators then being standardised on the CEGB system. Experience of the AGR was confined to the small demonstration reactor at Windscale, and the difficulties of extrapolation to larger sizes, later experienced also in the Magnox reactors, were not appreciated.

The reader of the book might infer that, in 1965, US experience was uniformly good, which it was not then nor always subsequently, whereas British experience was uniformly bad, which also was not true. It was in a different atmosphere from the one described by Duncan Burn that the Dungeness B assessment was made.

The CEGB procedures for tender assessments were well known within the Board and understood by those plant manufacturers who submitted tenders. It was the responsibility of specialist engineers, within the Board, to clarify any points of uncertainty in direct and confidential discussions with the plant manufacturers and, on the basis of their own calculations, to report at length on which submission best met the Board's needs. In the case of nuclear power stations the procedure had become more complicated by the concept of 'turnkey' contracts which had been introduced and, in the particular case of Dungeness B and contrary to the view expressed in the book, by the exceptional amount of detailed design data provided. It would have been impossible to proceed without very great help from the AEA, but the responsibility for a decision rested with the Board.

In chapter 10, it is stated explicitly that the assessment was biased and much of the blame is attached to the AEA, which is also accused of exerting excessive pressure on the CEGB. For example, on p163 it is said "in going AGR, the CEGB did so under the strongest pressure from the AEA". I cannot recall any meeting between the executive members of the CEGB and

the members of the AEA at which pressure was even suggested and, if within the assessment team itself, anything was done which was unreasonable, I would have expected it to be resisted strenuously. None was reported to the Board.

The conclusion was wrong for a very different reason. The extent of the extrapolation from the Windscale AGR to full scale was miscalculated and at Dungeness B, it was followed by some very bad engineering. Part of the explanation for the miscalculation must be attributed to the fact that the CEGB had inadequate strength in nuclear matters to arrive at a conclusion contrary to that of the AEA or even to differ appreciably from their opinions. This was a matter for concern in 1965 and it still is.

The usual purchasing arrangement between a public utility and a manufacturer of plants outside the nuclear fields is dependent on a dialogue between two approximately equal sides. This in the United States is roughly true for nuclear stations—also with little intervention from the national laboratories. In Britain, only fossil-fired power stations are treated in this way and the CEGB has an adequate engineering and R&D effort for a balanced discussion to take place, but in the nuclear field there is a single design and construction company—National Nuclear Corporation (NNC)—heavily criticised in the book which is dependent on the AEA, as is the nuclear fuel company BNFL and, in addition, the main R&D resources lie with the AEA. It is very difficult to say where responsibility lies and the CEGB is not strong enough to deal adequately with the technical problems of new systems, in direct discussions with the manufacturers.

It is this centralisation of technical ability in the AEA which Duncan Burn also sees as contributing to the inability of Government sensibly to take technical decisions, and this is discussed at length in the final chapter of the book. He would wish to see a competitive arrangement, based on American experience, with the AEA and the Government largely withdrawn from the discussions between the generating boards and the manufacturers. However, competition in the United States has reduced to only two firms to supply a large market. Probably only one could survive here, although not necessarily the present NNC. The Government cannot withdraw entirely, for, as the main source of finance for the building of power stations and for the development of nuclear stations, it is entitled to ask the questions which any prudent banker would ask. And, especially in safety matters but else-

where, too, there are political issues to consider.

The AEA position, as virtually the sole repository of information, will be changed with the recent decision to abandon the steam-generating heavy water reactor in favour of LWR and the main relationship might become direct between the generating boards and the overseas suppliers of LWR stations. But will it change very much if the board's negotiations have to be with the NNC and if the NNC is not free from the AEA? Even if the change is made, then, will it persist for the next Fast Breeder Reactor (FBR) the authorisation for which is long overdue, if it is to be available at the end of the century? Ought the CEGB to be happy with a situation in which its own R&D effort is concentrated largely on the operational problems of its already purchased reactors when it is

the generating boards who will need the FBR by the end of the century?

There are many other questions raised by Duncan Burn's analysis and one can only hope that the book will stimulate more urgent consideration of the answers needed. It is worth reading for this alone but there is much more that the reader will find interesting and provocative. The only serious criticism would be that the views expressed would have carried greater weight if they had been presented with greater balance and fairness. Even this is perhaps excusable, as it is a short book in which to cover everything on a big subject. □

L. Rotherham was Assistant Controller and Director of Research at the UK Atomic Energy Authority's Industrial Group from 1950-58, and a Board Member of the UK Central Electricity Generating Board from 1958-69.

Futurologists examined

Eric Ashby

World Futures: The Great Debate. Edited by Christopher Freeman and Marie Jahoda. Pp. 416. (Martin Robertson: Oxford, 1978.) £15.

IN 1973 the Science Policy Research Unit (SPRU) at the University of Sussex brought a refreshing voice of sanity into the overwrought and querulous debate about limits to growth. In a collection of essays called *Thinking About the Future*, H. S. D. Cole and his colleagues courteously and convincingly exposed the irresponsible prognostications of doom put out under the auspices of the Club of Rome. If SPRU had made no other contribution to contemporary thinking, this work alone would have justified their existence. The team has now produced a second book which, in my view, is even more important. It sets out to make a sober analysis of the views of 16 "major authors" who have published forecasts about the possible futures or fates awaiting the world as it moves into a phase of too many people, too little food, too dangerous energy, too attenuated mineral resources: Kahn, with his cocky optimism that all will come right (if you don't mind a few wars and some pretty extensive starvation) provided we stay on the path of *laissez faire* capitalism; Forrester, rattling the bones of a computer in the face of society; Schumacher, wistfully hoping that trans-national corporations will meta-

morphose into cosy little cottage industries; Heilbroner, who sees hope only in authoritarian government; Tinbergen, who believes that a genuine crisis will mobilise loyalty among people for the common good; Kosolapov, whose Marxist optimism brings him astonishingly close to the capitalist optimism of Kahn; and 10 others: Spengler, Ehrlich, Meadows, Dumont, Mesarovic, Leontief, Kaya, Herrera, Modrzinskaya and Galtung.

Christopher Freeman and Marie Jahoda have organised their material in a bold and successful way. They propose that the beliefs of the 16 authors whose work they consider, and the beliefs of their own team (by no means unanimous) can be roughly—and they emphasise that it is roughly—classified into three categories: conservative, reformist and radical; each with a distinctive "world view" which sets boundaries to the assumptions each author makes about possible futures. Thus, the radical may regard revolution as a necessary prelude to a future equilibrium; the reformist would favour big, but incremental, changes; and the conservative would question whether the background needs to be changed at all. They then make an equally bold generalisation about possible futures. Wisely they eschew computer scenarios which, as they say (p70), "disguise rather than reveal the extent to which their results are dependent on their assumptions and data". Instead they propose four options: high growth accompanied by a persistence of the present inequalities between richer and poorer nations; low growth, also accompanied by the present inequalities; high growth accompanied by a major and persistent narrowing of the gap between rich and poor; and low growth, also accompanied by a narrow-

ing of the gap between rich and poor. They then examine, in impressive detail, the possible consequence of these four options as they are perceived by conservatives, reformists and radicals. It is a skillful discussion of the interaction of three different political attitudes on four different possible futures for society. It is a simple framework on which facts and ideas can be attached, quite the most useful one which has yet been designed. In her eloquent conclusion to the book, Marie Jahoda sets out its three aims: to make a contribution to forecasting methodology, to take a substantive position in the world futures debate, and to offer some ideas for considering deliberate actions and choices in the less distant future which may affect the fate of the next two or three generations. They are modest aims, and all three of them are achieved.

No one is competent to take part in this 'great debate' who has not mastered the relevant facts about forecasts for population, food energy and mineral resources. These facts are set out in four introductory chapters, which cover familiar ground, although some of the conclusions drawn from the facts will be challenged. No one will dispute the recklessness of making predictions about population: the estimates by the 16 futurologists differ by almost an order of magnitude! Over food supplies the authors conclude that the limiting factor is not the amount of food which can be produced; it is the lack of means to distribute the food equitably and the lack of money in the Third World to pay for food. This view will be disputed by some experts, although no one is likely to underestimate the disgraceful mismatch between butter mountains in one part of the world and starvation in another. Over mineral resources the authors are equally optimistic: there will be shortages of specific materials but—in their view—these shortages will not be likely to have a desperate impact on the quality of life.

The Achilles heel for a stable future, the authors say, is energy. If there is not a sufficient supply, all else—food, industry and peace—is put at risk. If a sufficient supply is ensured by massive development of fast breeder reactors and the like, then other dangers which have had plenty of publicity—what to do with wastes, what to do with terrorists—take the place of the dangers due to insufficient energy. The authors favour a eclectic solution, with much more attention than there is at present being paid to energy from the sun, wind and tides. Again, some experts will challenge this view as unrealistic.

Differences of opinion over the content of these chapters do not, however, affect the main argument of the book,

which is that the first symptom of crisis over food, energy, or resources will not be physical shortages of these things: it will be social and political disturbances; and it is to these that emphasis should be given, and given now.

The important and original part of the book carries a set of sober speculations. The authors make a list of seventeen features which play a part in determining the quality of life of ordinary citizens. The list includes intangibles, such as geopolitical issues, and tangibles, such as food, health, transport and energy; and they add to these features the two emotive words which have drawn men into war and revolution for millennia: liberty and equality. How would conservative, reformist and radical regimes act toward these emotive words and the seventeen parameters for the quality of life in conditions of high or low economic growth, and at greater or lesser levels of equality? Into this matrix of three world views $\times$ four conditions of growth and equality $\times$ seventeen features contributing to quality of life (204 in all) the authors pack their conjectures for possible futures. The matrix covers thirty-four pages; it contains, in my view, more practical common sense (which is the attribute which matters most in the Civil Service or in Government), and more credible assumptions (which are the most important ingredients for sound economic advice) than I have read in any other document about forecasting. Of course, it makes sombre reading. High economic growth continued indefinitely leads to the absurdity of all unconstrained exponentials. Low economic growth continued indefinitely leads to frustrated expectations, and these in turn lead to conflict. Perpetuation of the present inequalities between rich and poor nations are a recipe for revolution and war. Reduction of present inequalities—even with some growth—makes demands for sacrifices from affluent nations which, so far, have met with a derisory response. (The world's annual expenditure on armaments is some 15 times more than the aid from affluent to third world countries.) Struggle, turmoil, revolution, stagnation: these are words which appear unpleasantly often in the matrix.

But the SPRU team, despite ideological differences among them, do come to a firm conclusion. Of the four options they propose, the one which offers the least dangerous prospect for mankind is the scenario for high growth and more equality. The conservative would hope to attain this by creating a larger cake of wealth so that poor nations could share it; the reformist would expect affluent countries to react

only under threat to world stability, and then, no doubt dragging their feet, to make sacrifices so that poorer countries could 'leapfrog' into appropriate levels of industrialisation. The radical would rely on the seizure of political power by the workers, followed by a gesture of altruism (not apparent among workers at present) toward their poorer brothers in the third world.

The outstanding practical conclusion from the analysis is that the absolutely top priority, the need which should take precedence over all other needs, is to narrow the gap between rich and poor, both within and between nations. This is not an original sentiment; but the authors of this book do not just offer it as a pious hope. They press the point: that the way to reduce inequality is to devote massive resources to problems to which the affluent nations are paying very little attention: research and development (for instance) on tropical agriculture and on intermediate technology. They stress, too, that innovation is needed far more urgently in political institutions than in technology. Anyone who reflects on the problems which lie ahead is brought up against the ugly prospect that the problems may not be soluble under a pluralistic democratic government. The alternatives are one or other version of authoritarianism. Is it practicable to devise an authoritarian pattern of government which requires obedience over principles (such as is already demanded by the Highway Code in the UK) but leaves individuals freedom (as motorists in the UK indeed have) to exercise their liberty (for example, when driving, provided they comply with the code)?

The high level of generalisation in this book constitutes its strength. Indeed, the authors themselves repeatedly remind the reader of the futility of specific technology assessment. Discontinuities make nonsense of such assessments. For instance, the National Resources Committee of the Department of the Interior in the USA made a thorough prediction of technological trends in 1937. They failed to forecast atomic energy, computers, radar, antibiotics, and World War II and its social consequences: no-one can blame them for these omissions, but this example, and scores of others like it, should make anyone sceptical about similar specific forecasts being made today. The paramount potential discontinuity, of course, is the finite possibility of a nuclear war. In a heroic chapter, the authors of this book tackle this problem too. And it leads them to a sombre conclusion: namely that a policy of disarmament among the great powers (which would entail a cessation of activities costing over $\$200 \times 10^9$

per annum) would itself precipitate a major social and economic upheaval.

In a world deluged with books, many busy people have to content themselves with reading the reviews and not the books themselves. It would be a great mistake to read only the reviews of this book. It is essential reading for anyone

who wishes to participate in the debate about man's future with (as the authors put it) "informed imagination". □

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Détente and dissent

Robert Lewis

Soviet Science. By Z. A. Medvedev. Pp. 262 (Norton: New York; 1978.) \$10.95.

ACCORDING to the 'blurb' on its cover, Zhores Medvedev's latest book is

"a brief history of science in the Soviet Union with an emphasis on the interaction between scientific and technological developments and the political goals of the Soviet government".

However, the potential purchaser should not be misled by this statement, for as the author himself admits in his introduction, this is not a book for those who wish to have a short and reasonably comprehensive account of the development of science and science policy in the Soviet Union since 1917. Indeed the years between 1917 and 1953 are summarily dealt with in five short chapters and a little over fifty pages. Furthermore these chapters contain several errors. For example, the author states that in the years 1922-28 science was directed at the government level by the People's Commissariat of Education, when, in fact, this commissariat was only one among a number of government organs involved in science policy-making and was becoming less important in this field. These initial chapters are padding which make the manuscript of better length for a book and which the publishers presumably hope will increase its marketability.

The real heart of Medvedev's book is an essay on science in the USSR under Khrushchev and Brezhnev with particular emphasis on the two themes of *détente* and dissent. The author restates his view that *détente* has not been a consequence of Soviet military weakness and is not a necessary condition for the continuing development of Soviet military technology. On the other hand he considers that it has had important consequences for Soviet scientists working in non-secret fields, bringing an acceleration in the renewal of links between Soviet and world science which had begun under

Khrushchev. More Soviet scientists have travelled abroad and more articles have been published in foreign journals; the maintenance of informal contacts has become easier. This increased contact has strengthened the feeling among Soviet scientists that they, like scientists elsewhere, are participants in a universal scientific culture which is independent of any particular set of national values. This degree of cultural autonomy, reinforced by the high prestige which scientists have in Soviet society, has put them in a special position. Indeed, the author reports a conversation which he had with Kapitsa in 1967 when the latter suggested that the function of the United States' Supreme Court and the British House of Lords as independent arbiters in constitutional problems seemed to be falling morally on the Academy of Sciences of the USSR. These special conditions surrounding Soviet science are seen as major factors in the relatively high frequency of political dissent amongst scientists. Medvedev reviews the policies adopted by the Soviet government and the

Communist Party to counter dissent by such means as the exercising of closer control over the award of higher degrees and promotion with the aim of preventing dissidents rising higher in the scientific hierarchy; he is, however, sceptical of the effectiveness of such measures.

Thus, the Soviet government, as the author points out, is in a dilemma. On the one hand the future progress and prestige of Soviet science, to which the government attaches great value, depends on international contacts—a simple instance is that, as a result of lengthy publication lags in Soviet journals and of the inability of most other scientists to read Russian, publication abroad is likely to be needed in the vital matter in modern science of establishing priority. On the other hand the closer attachment of Soviet scientists to the international scientific community and its norms and values increases their degree of cultural autonomy and consequently the problems of political control. The author does not offer any firm prediction of the likely outcome of this clash of values, but he does foresee the possibility of future new waves of open dissent and that these could be stimulated rather than eliminated by attempts to control science and scientists more closely. □

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Soviet technical intelligentsia

Julian Cooper

Technology and Society under Lenin and Stalin: Origins of the Soviet Technical Intelligentsia, 1917-1941. By Kendall E. Bailes. (Princeton University Press: Princeton, New Jersey, and London, 1978.) £20.10.

THE role of engineers and other technical specialists in a society in transition between capitalism and socialism is a question which, for understandable reasons, received little attention in the writings of Marx and Engels. In a rarely cited letter of 1890, however, Engels wrote:

"Of course we still have insufficient technicians, agronomists, engineers, chemists, architects, etc., but if the worst comes to the worst we can buy them for ourselves, just as the capitalists do, and if some of them turn out to be traitors

(as will certainly be the case in that society) they will be punished for the edification of the others, so that they will appreciate that it is not in their own interests to rob us any more. But with the exception of these specialists, and also the school teachers, we can very well do without the rest of the 'educated'. For example, the current strong influx into the Party of penpushers and students brings all kinds of trouble in its wake, unless these gentlemen are kept within proper limits".

Lenin, in the years preceding the October Revolution, also found such 'gentlemen' troublesome, but immediately after the successful winning of power the Bolsheviks had to face up to the problem of retaining the desperately needed skills of the technical specialists, the overwhelming majority of whom were unsympathetic or actively hostile to the Soviet power. In his pioneering study, Kendall Bailes, associate professor of history at the University of California at Irvine, is concerned with the fate of these engineers and technicians and the contradictory process by which a new technical intelligentsia, in the main loyal to Soviet society, was formed. The relationships between the technical intelligentsia and other social groups are

explored and an assessment made of its place and role in the political system during the formative years of the modern Soviet state.

As Bailes lucidly relates, in the early years under Lenin's insistence and in the face of opposition within the Party the Bolsheviks did indeed 'buy' for themselves the services of the bourgeois technical specialists, both by ensuring them relatively privileged material conditions during the lean years of civil war and famine, and also by providing improved opportunities for applying their professional skills. In the very first years of the revolution a number of important new applied research institutes were founded and many engineers, though unsympathetic to the regime, were involved in the drawing up of the famous GOELRO plan for electrification of 1920.

At this time, as Engels predicted, 'traitors' were punished for the edification of their colleagues. By this carrot and stick approach the Party succeeded in winning the majority of the old technical specialists to work for the Soviet power, and some forsook their political antipathy or neutrality and joined the Communist Party. From 1928 to the summer of 1931, at the time of the adoption and initial implementation of the ambitious first five-year plan and the associated struggle against the right-wing opposition led by Bukharin, a section of the old technical intelligentsia came under attack, accused of 'wrecking' activities. These events are described in detail by Bailes, who argues with originality and plausibility that the Party leadership around Stalin feared the growth of a 'technocracy' movement which, like its counterpart in the United States at that time, sought to elevate the engineers as a professional group to a position of political influence and power.

In order to break the country's dependence on technical skills without socialist commitment, the Party launched a large-scale programme for the education of a new generation of Soviet engineers, the majority of whom were of working class origin. Bailes devotes considerable attention to the formation of this new technical intelligentsia and chronicles its expansion and mounting influence during the 1930s. In the purges the technical specialists, like other professional groups, suffered greatly, although Bailes is probably correct in suggesting that engineers and technicians had a higher survival rate than, say, the industrial managers. Even after the worst waves of repression many representatives of the old technical intelligentsia continued to occupy posts of authority in industry and higher education. Before and during the purges Stalin and other Party leaders constantly stressed that

specialists had to be politically conscious and committed, and it is notable that by 1937 there were almost 50,000 Party members with higher technical education, compared with a mere 138 graduate engineer Party members in 1928.

The rapid economic and social development of the pre-War decade was accompanied by a radical transformation of the political status of the engineers: they started as critical outsiders and ended as a significant group within the Party and government. Between 1939 and 1941 no less than 70% of new Party members came from the ranks of the administrative and technical intelligentsia. As Bailes observes, many of the members of the present-day Party Central Committee and Politbureau, including Brezhnev himself, are representatives of this new generation of technical specialists who embarked on their careers in the turbulent pre-War years.

In discussing the origins of the Soviet technical intelligentsia (throughout inelegantly and inaccurately termed the 'technostructure'), Bailes draws on an impressive range of sources, both Soviet and Western, and presents much new interesting material. Rejecting both the totalitarian and the more recently fashionable interest groups models of Soviet politics, the author adopts a more flexible approach, placing greater emphasis on the interaction of social forces making for cohesion and conflict than is usual in discussions of the Soviet Union in the 1930s. This leads Bailes to analyse political developments between 1931 and 1937 in terms of a growing tension between the industrial managers and technical specialists and their spokesmen in the Party leadership on the one hand, and the group of 'Stalinists' (not satisfactorily defined) on the other, with the planning process as the focus of discord. But this interpretation, central to the author's discussion of the purges, is not wholly convincing. The planning

process is seen one-sidedly in terms of the setting of output targets, to the neglect of problems of the allocation of inputs, which at times produced quite different conflicts of interests. In general, the author's grasp of economic and, in particular, technical matters is not as sure as his understanding of the processes of social and political changes, although in analysing the latter a debatable definition of class is used, according to which manual and non-manual workers, including the entire technical intelligentsia, form two distinct classes.

With a work of such impressive thoroughness and length it is perhaps unreasonable to point to omissions, but one would have welcomed more consideration of the substantive technical issues which were debated by the engineers, often with great passion, during the years of rapid industrialisation—for example, the relative merits of American mass-production engineering technology as opposed to the traditional Western European methods. There is also insufficient consideration of the important contribution of foreign technical specialists (over 9,000 were working in the USSR in the autumn of 1932) and a related tendency to divorce Soviet internal developments from events in the then increasingly hostile outside world: it could be argued, for example, that the moves in the summer of 1931 to improve relations with the old technical intelligentsia were not unconnected with the measures in May to reduce the level of reliance on foreign technical assistance.

Kendall Bailes has provided an important contribution to the steadily growing body of literature on the history of Soviet society which seeks to take a fresh approach based on serious original research, rejecting the crudities and prejudices sadly prevalent during the Cold War years. □

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History of integrated circuits

Andrew Holmes-Siedle

Revolution in Miniature: The History and Impact of Semiconductor Electronics. By E. Braun and S. MacDonald. Pp. 231. (Cambridge University Press: Cambridge and London, 1978.) £8.50.

"AN integrated circuit is a truly awe-inspiring achievement of human

inventiveness". So say the authors of this book. Those working on silicon technology, who know what effort and physical insight is packed into those minute structures, will not find it difficult to agree with this statement. For those not so privileged, the integrated circuit is likely to inspire puzzlement rather than awe. The structures concerned consist merely of microscopic modifications on the surface of a silicon chip. They usually lie sealed in anonymous little ceramic or plastic blocks and these themselves are commonly shut away inside the case of a calculator, television set or satellite. Hardly stirring stuff for the average man. How can one bring home to the layman the

undoubted revolution which they are bringing about in industry and the home?

The authors of this book, who are members of the Technology Policy Unit at the University of Aston in Birmingham, have made an attempt to view the history of the integrated circuit as a whole and bring a little of its flavour across to the intelligent reader. They have tried to add a little life to that formidable array of technological fact in several ways: first, by conducting interviews with some of the engineers and physicists involved and quoting parts verbatim—this is very successful; and second, by adopting a loose form of comment which one can only call journalese (“for a fraction of the cost of one week’s housekeeping, one can have access to any number’s square root”). The results are sometimes fascinating but often baffling, especially when sweeping statements are made without support and the references, when one turns to them, are more often to *Business Week* than to the original literature. Perhaps this is the style of theses in technology policy but it is galling to a serious reader who is reaching for his pencil in anticipation of an original historical reference which he can follow up. Perhaps this is asking too much, however, of a book which the authors direct to the “intelligent lay-reader”. Nevertheless, there are at least 600 references given. This makes the work a curious mixture of academic study and “pop history”.

The opening chapters of the book are well worth reading, being an enjoyable and brief account of the history of the invention of the transistor, with suitable comments on the influence of science, commerce, the military and the upheavals caused by World War II. The hothouse atmosphere of Bell Laboratories is well created and the subsequent commercial spread of the transistor is dealt with in fairly conventional narrative form. The authors avoid any diversions into technical explanation, possibly leaving a little too much for the general reader to pick up elsewhere. It is noteworthy that the only line drawings in this book are financial charts. How many scientists could write a book without recourse to technical sketches?

In this section, historical balance is lacking in at least one respect: the invention of the metal-oxide-semiconductor (MOS) transistor by Hofstein in 1962 is passed over too lightly. Although the events of the late 1940s at Bell are exhaustively documented (at least 60 references are quoted including dozens of interviews), the inventor of the MOS device is neither named nor interviewed. The term “MOS” is only mentioned a few times in the whole book and this can be viewed as a

serious lack of balance, because the ability to manufacture large-scale integrated circuits cheaply from MOS elements has been the main driving force of the revolutions of the 1970s. It is also predicted by others that MOS circuits will dominate microelectronics in the 1980s. In fact, the history of the Intel company, the manufacturer of the first MOS microprocessor, is probably as important as that of Bell, but it is not described except as part of a more general description of Silicon Valley.

The later chapters of the book bring the reader up to date concerning the invention of the integrated circuit and the immense expansion of uses which came with this step. More space is given in this section to business considerations, such as figures for the growth of markets and the pricing of components. It is likely that the technologist will find this more interesting than will the lay-reader. It is also here that the very speed of technological progress which all commentators emphasise seems already to have dated the book. Electronic games, Viewdata and the charge-coupled device are not mentioned, and the word “microprocessor” is not even listed in the index. The splendid compatibility between the integrated circuit and the satellite is not noted and Space is, in fact, only

mentioned as part of an “awesome list” of weapons. These, the authors point out, are “packed with electronics from top to bottom”. It is in this section that oral history techniques come into their own. The interviews give the right tang of “entrepreneurialism” (*sic*) flavoured with physics which characterises the microelectronics industry in the USA. The authors should certainly persevere with this method.

The concluding chapter presents “Reflections of an Electronic Age”. From authors who have performed a social analysis of the past three decades of electronics, one expects some penetrating statements covering future applications and the future cultural influences of microelectronics. This the authors attempt to do but the statements too often dissolve into generalisations that are far from penetrating. It is not too much to ask even a popular history book to be penetrating.

We badly need help in understanding the wide social impact of microelectronics. This book may help many by its factual survey but, for this reader, it provided no more than the faintest glimmer of fresh understanding. □

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What use is astronomy?

A. J. Meadows

Man and the Stars. By Hanbury Brown. Pp.182. (Oxford University Press/Clarendon: Oxford and New York, 1978.) £5.95; \$14.95.

PROFESSOR Hanbury Brown explains in the preface how he came to write this book:

For fifteen years I have run an astronomical observatory in the Australian Bush . . . In that time I must have shown the place to thousands of visitors and tried to explain what we were doing and why . . . However, there was one particular question which always worried me because I couldn’t answer it satisfactorily in the time available, usually only a few minutes before the coach left. It was often asked tentatively, away from the main group of visitors, as though it might be silly or, perhaps, impolite. It was always the same basic question—“This work may be competent and interesting, but is it any use?”

I found it comparatively easy, given a few minutes, to persuade people that our work was relevant to the general

study of astronomy, but I have never been able to abbreviate, to my satisfaction, the arguments that astronomy is an integral part of science and that science is an essential part of our civilization. And so, as the coaches vanished in a cloud of red dust, I was often left wondering what the visitors really thought of our observatory. Many of them, I suspect, felt much the same as they would have done after a tour of a monastery—interesting maybe, but a useless way to spend one’s life!

What this book sets out to do, therefore, is to try and sketch out Professor Hanbury Brown’s own answer to the question—what use is astronomy? It is a question that should engage the attention not simply of astronomers, but of all pure scientists. Most of us have our salaries, our accommodation and our equipment provided by the state—that is, ultimately, by everybody else in the community. How can we justify this expenditure to them?

Here, for astronomy, we are offered answers under five headings: stars and the calendar; stars and time; stars and navigation; stars and science; stars and man. (I am not altogether clear why the title is *Man and the Stars*, even though the chapter headings run in the opposite direction. Perhaps it is to remind us that astronomy could not be practised if either of the two components ‘man’ or ‘stars’ were missing.)

The purpose behind each of these headings is to emphasise how the development of astronomy and the growth of other human activities and thought interact. From this viewpoint, the first three chapters should really be read together. They recount how man down the centuries has used astronomy as a means of fixing his position in time and space. This ability is obviously fundamental to the existence of civilisation. Agriculture and religion needed a viable calendar; organisation of the working day required good time-keeping; travel and, therefore, commerce over any distance demanded accurate navigation. The fourth chapter tackles a different aspect of astronomy: its role in the advancement of basic science and, in particular, physics. The final chapter looks at the much more diffuse and difficult question of the influence astronomy has had on the way we interpret and experience the world around us.

We are provided with a well written and lucidly argued case for the importance of astronomy, but I feel that somehow, for much of the book, the author actually avoids the main point. The real question is not whether astronomy had immediate relevance to everyday life in times past, but whether it has such relevance today. Already in the eighteenth century, astronomy was ceasing to be of practical concern to more than a few people. Thus, the astronomer in Samuel Johnson's *Rasselas* says that he has devoted his life to the wrong subject, because the knowledge he has acquired is of little use to mankind. Of course, astronomy still has some practical uses today. Some years ago I was telephoned by a major British company, who required a quick astronomical method of checking some alignments in their surveying operations. I suggested a method which was adopted—so, is this not a clear example of a practical use of astronomy? (As I forgot to ask for a consultancy fee, it unfortunately does not simultaneously demonstrate the practical use of astronomers.) But the concepts behind my proposal had been known for centuries. The problem is that the ideas most astronomers are concerned with today seldom, if ever, have relevance to everyday affairs. I find the counter-examples that Professor Hanbury Brown suggests distinctly unconvincing. For example, he mentions the importance of astronomy for space navigation. But this simply raises the question—what is the current utility of space navigation? The only space activities which certainly have a value for everyday life are those concerned with Earth applications, and the satellites involved in these hardly require space navigation.

It is worth laying considerable stress on astronomy's lack of immediate utility because it distinguishes astronomy from other major divisions of science. The others can all claim to have something to contribute towards the material interests of human beings; but astronomy has declined over the past few centuries from being a highly useful subject to being almost useless. Nor has astronomy provided much basic information to other areas of science. Rather, throughout the twentieth century, astronomy has taken more from other disciplines than it has given to them. Yet astronomical research has not declined over this period. On the contrary, state support for astronomy is now provided on a larger scale throughout the world than would have been imagined possible a century ago.

If we seek a reason for this funding it must derive then from the importance of astronomy for our view of the world. This has continued to be recognised long after the practical value of

astronomy declined. Thomas Huxley, presumably unbiassed as a biologist, claimed that astronomy is the science "which of all sciences has filled men's minds with general ideas of a character most foreign to their daily experience, and has, more than any other, rendered it impossible for them to accept the beliefs of their fathers". As long as this sort of claim can be made for astronomy, or for any other branch of pure science, it can surely hope to receive financial support. But there is a proviso. For astronomy to affect our view of the world, its advances must be made available and understandable to people who are not astronomers. Professor Hanbury Brown therefore concludes his book with a plea for the proper popularisation of science. He is to be congratulated on practising what he preaches in such a readable way. □

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Technological day-dreams

R. L. F. Boyd

Spaceships of the Mind. By Nigel Calder. Pp.144. (British Broadcasting Corporation: London; Viking: New York, 1978.) £6.50; \$14.95.

WHEN a project in space science or technology is undertaken in the real world, the lives and reputations of real people are at risk. Real public money is spent for which there are many competing calls: many of them are less worthy, but some may have stronger claims on resources. Those involved in the decisions and execution of the projects are usually alert to the glare of publicity surrounding their activities and the incalculable consequences of failure. The long road to realisation involves a succession of studies followed by detailed scrutiny and by battles for authority to proceed, which may be lost or won. A typical sequence involves Mission Definition, Pre-feasibility Study, Feasibility Study. These are sometimes called Phase A, may cost several per cent of the final project and usually have a significantly less than even chance of surviving for further work.

The next phase — Phase B — the Design Definition, involves a hard and disciplined engineering grind in which 'state-of-the-art' parts of the project are carried through to a point of

readiness when a "metal-cutting" realisation of the project — Phase C — can be or perhaps cannot be entered upon. Any parts of the project requiring research and/or development will have been identified, assessed and costed. Some limited experimental work such as determining the emissivity/absorptivity of a particular surface material or its deterioration by solar ultraviolet radiation may have been undertaken. Exceptionally the procurement of long lead items not representing a major part of the cost may have been put in hand. By this time 10% or so of the total cost to launch will have been committed. That cost will have been established to within about 10% and a decision whether or not to proceed must be taken.

Phase C, the production phase, is a long agony of finding out how difficult things are in practice; fighting specification deviations (one firm supplied me with stainless steel screws made, we discovered in the final magnetic survey of an Orbiting Solar Observatory, from "the nearest alloy to the one specified which was available"; they were excellent in all respects except the one that mattered most; they were magnetic); fighting safety problems real and in the minds of ill informed safety officers; testing, going back to the drawing board, re-testing, accepting or rejecting compromises and so on.

Spaceships of the Mind make no such demands on reality but like other hallucinations are probably best regarded as a (bad?) dream. I would have settled for science fiction but the necessary imagination and drama are

absent. Calder's previous book *The Key to the Universe* (but not the television production) was, in my view, a brilliant service to science. It showed to the intelligent layman such coherence in our understanding of the mystery of being as could be shown and my only quarrel was with the suggestion that there is likely to be a key to so much that could be grasped in any ultimate way by the limited system of neurones in the human brain.

Unfortunately this latest book (mainly about space colonisation) fails to convey the nature of the process by which technical problems are specified and solved, thus obscuring the gap between the dream and reality. I have theoretical astrophysicists on my staff but when I want to know what can or cannot be done for a projected payload I go to my engineers.

Calder goes to distinguished scientists of which the late J. D. Bernal is the prototype. He talked to John Lewis a cosmic chemist, to Freeman Dyson an eminent theoretical physicist, to Sol Spiegelman a distinguished molecular biologist, to Stephen Cheston an historian, and to many others of comparable distinction and similar qualifications. He did talk to some engineers. The late Val Cleaver was one of the greatest, and he was inclined to think that a "distinguished physicist" like O'Neill "ought to know better" than to suggest space colonisation on a time scale of decades. Theodore Taylor was a rather different engineer whose ideas entitled by Calder "The Santa Claus Machine" seem to me as far from any realistic materials science as that childhood myth. I cannot believe that justice(?) has been done to this former Princeton professor. I feel the same about Henry Kolm and his "mass driver"—an electromagnetic gun for ejecting pieces of asteroids or the Moon or what-have-you either for reaction propulsion or as a kind of space parcel post. These engineering essays carry no conviction because they are presented with no calculations. This is a disservice. Above all else, physical science and engineering is numerate. If this lesson were learnt at school we would be spared the correspondence from cranks who invent this or discover the explanation of that, when a sum on a postage stamp would expose the nonsense.

On the credit side, Calder does devote some attention to the Earth which in all conscience needs it more surely than the rest of the galaxy; he shows some recognition that ethical problems of humanity may overwhelm the day-dreams and he makes it clear that his sample of prophets has been strongly selected to "test the hypothe-

sis that recent advances in scientific knowledge ought to have stirred constructive thoughts". The hypothesis is verified. What other thoughts will be stirred for good or ill I cannot guess. If you are only going to buy one book buy *The Key to the Universe*. If you are going to buy two let me commend a story Dingle once told before a lecture by Eddington on his more re-

condite and less certain speculations. A man went into a restaurant and asked for a boiled egg and a kind word. The waitress brought the egg and turned to leave the table. He reminded her he wanted a kind word. "Don't eat it", she said. □

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Rebel without a cause

Michael Rowan-Robinson

Scientists Confront Velikovsky. Edited by Donald Goldsmith. (Cornell University Press: Ithaca, New York, and London, 1978.) \$8.95; £5.95. *Velikovsky and Establishment Science*. Edited by L. M. Greenberg. (Kronos: Glassboro, New Jersey, 1978.) \$9.95.

IN 1950 a remarkable book by a Russian born psychiatrist, Immanuel Velikovsky, who had lived in the US since 1939, was published. The book, *Worlds in Collision*, argued that the biblical account of the Israelites' flight from Egypt, together with numerous other ancient texts from cultures all over the world, carried the record of great natural catastrophes. Venus, ejected as a comet from the planet Jupiter, passed close to the Earth, causing earthquakes, volcanoes, floods and plagues, then dislodged Mars from its orbit onto a near collision course with Earth, before settling in its present circular orbit. The book aroused fury amongst certain members of the astronomical establishment. Efforts were made to prevent publication of the book, and to persuade astronomers to boycott the publishers.

Gradually the furore subsided. The book has been popular with the public but has for the most part been ignored by astronomers. Velikovsky is an excellent writer and if he had taken the trouble to learn what is known in astronomy and geophysics, he would have made a wonderful populariser of the field. As it is the book is of little scientific interest. None of the ideas are developed in any quantitative detail. The "predictions" are of the type: Venus is hot, Jupiter will be "a source of radio noises", the polar caps of Mars are "of the nature of carbon", argon and neon will be present on Mars "in rich amounts". Velikovsky and his supporters make great play of the cases where these "predictions" fit subsequent observations, but no effort is made to confront the theory as a whole with what is known.

In the early 1970s amid the growing popular interest, particularly in the US, in offbeat ideas and cults like parapsychology, the occult, astrology, and UFOs, Velikovsky became something of a guru. Two publications, *Pensée* and *Kronos* were devoted to his ideas. The young and rebellious flocked to his banner. Once again the establishment became alarmed. At the February 1974 meeting of the AAAS in San Francisco, a symposium was held at which four scientists tried to confront Velikovsky with the brick wall of facts which his theory must run into. One eye-witness told me he felt sorry for the way this old man was being utterly demolished by these arguments. Yet in his reply, Velikovsky steered his way round the wall to the satisfaction of himself and his vocal supporters.

It is unfortunate that *Scientists confront Velikovsky* contains only the arguments against Velikovsky, for it reads like Hamlet without the Prince. Velikovsky's contributions, together with nine other articles by his supporters, appear in a separate book, *Velikovsky and Establishment Science*. The reason for this fragmentation of what ought to have been the *Velikovsky Symposium Proceedings* is that Velikovsky asked for more space to answer his tormentors than the editor was willing to allow. To me this seems a piece of unnecessary pettiness. In the event Velikovsky's contributions amount to rather fewer words than those of his principal inquisitor, Carl Sagan.

It has to be said that Velikovsky faced his ordeal with considerable dignity, both at the symposium itself and in his published articles. This is not always true of those whom the scientific establishment rejects and despises. In some, like Herbert Dingle in *Science at the Crossroads*, the bitterness of their treatment becomes more important than the scientific case they are trying to make. This type of attitude is prevalent in many of Velikovsky's supporters. The main object of their hate is Sagan and they have worked very hard to ferret his more eccentric speculations out of the literature, with some success. Why should Sagan be allowed to speculate about life on Mars, extra-terrestrial civilisations and "galumping beasts" out there, and then ridicule

Velikovsky's notion of fly larvae in the tails of comets? More generally, why is it OK for Hoyle and Wickramasinghe to speculate about world-wide epidemics brought by cometary dust, and for Sagan to speculate about the evolution of human intelligence, but not OK for Velikovsky to speculate about astronomy?

The crucial point, to be borne in mind by all weekend cosmologists, is that a demonstrated awareness of what is known and what current ideas are is a prerequisite to being listened to seriously by scientists. The more you have demonstrated that you know, the more your speculations will be listened to. If you are Hoyle or Ryle, your views on energy production will be listened to with sympathy. If you are Einstein, your views will even be sought on the international political scene.

How good a demolition job did Sagan *et al.* do on Velikovskianism? Although some of them, particularly Sagan and Huber, obviously put a lot of work into preparing their contributions, the result is not altogether satisfying. Rather too many weak arguments, niggling at peripheral issues, appear in these pages. The most solidly argued case is that of David Morrison (not actually a symposium participant) who doesn't bother to chase every hare Velikovsky starts. He concentrates on the solid bedrock of facts that Velikovsky is inconsistent with; the huge difference in composition between Venus and Jupiter, and between the allegedly interchanged atmospheres of Venus, Mars and Earth; the evidence that the era of major cratering on Mercury and the moon ended 4×10^9 yr ago, rather than 3,000 yr ago as Velikovsky believes; the evidence that the moon has never been molten, so that craters cannot have formed by bubbling; the evidence from the craters of Venus that it cannot have been torn from Jupiter recently; and the absence of evidence for residual excess heat in Venus and Mars.

Sagan has additional arguments involving the difficulty of ejecting Venus from Jupiter; the low probability of cometary encounters with Earth; the difficulty of restarting the Earth's rotation once it has been stopped; the problem of circularising the orbit of Venus; and the total mass of manna required to feed the children of Israel for 40 years. Apart from the manna question, which I find a bit of a red herring, these difficulties do not seem to me insuperable given the initial catastrophist framework. Similarly Huber's demonstration that the ancient Babylonian Ammisaduqa cuneiform tablets are consistent with the modern orbit of Venus is fascinating, but hardly compelling evidence against Velikovsky in the light of the data

message, not to say downright resuscitation, required.

The arguments of '*Scientists confront Velikovsky*', though of variable quality, are overwhelmingly strong enough in totality to reject Velikovsky's scenario. Why then do the young and rebellious, not to mention a few professors of history, philosophy and anthropology, follow Velikovsky? Asimov, in an entertaining forward to the anti-Velikovsky book, suggests: "Supply the public with something amusing, that sounds scholarly, and that supports an idea it wants to believe (in this case the literal truth of the Bible), and surely you need nothing more."

Does anything then remain of Velikovskianism? What is the basic core of his idea? That a comet passed near the Earth in classical times causing chaos. Suppose a massive long period comet were first noticed towards Jupiter and then appeared as a bright

evening or morning star before brushing past the Earth. Would not the ancient myths then speak of Venus, daughter of Jupiter, bringing disaster? Stripping Velikovsky's scenario of the direct association with Venus and Jupiter eliminates almost all the refutations offered against him. It also, of course, eliminates almost all Velikovsky's astronomical "predictions". What would remain is his evidence from the ancient texts, easily the most interesting part of *Worlds in Collision*. Can such a scenario be refuted? And if it were one day proved correct, would we say that this curmudgeonly old rebel had a cause after all? □

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Ptolemy in the dock

D. T. Whiteside

The Crime of Claudius Ptolemy. By R. Newton. Pp.411. (Johns Hopkins University Press: Baltimore and London, 1978.) £15.75.

"THIS is the story of a scientific crime", begins the author, "committed by a scientist against his fellow scientists, a betrayal of the ethics and integrity of his profession that has forever deprived mankind of fundamental information about an important area of astronomy and history". And he concludes his summing-up for the prosecution: "All of his own observations that Ptolemy uses in the *Syntaxis* are fraudulent, so far as we can test them . . . His work is riddled with theoretical errors and with failures of comprehension . . . His models of the moon and Mercury conflict violently with elementary observation . . . The *Syntaxis* has done more damage to astronomy than any other work ever written, and astronomy would be better off if it had never existed." In between, for almost four hundred pages, comes a relentless attack on the character and achievement of "the most successful fraud in the history of science" such as would do any cross-examining QC in the Old Bailey proud.

Shifty-faced Ptolemy in the dock is found morally guilty of systematically falsifying past records for his own ends

and left with only the odd one of his own observations of the heavenly motions which "may be genuine" (never, of course, the charity of "may be false". . .). He is not allowed credit even for his bissextile equant model of circular motion for the outer planets and Venus—that on which alone a near-perfect theory of all the solar planets may be founded, and which even Newton must allow to be "indeed . . . a contribution to astronomy". "There is no evidence", he thunders, "that [its discovery] was made by him . . . and Ptolemy lessens its value considerably by his inaccurate use of it". What a dullard lot succeeding astronomers have been—Copernicus especially (on whose debilities Newton is almost equally severe)—never for the best part of the next two millennia to have suspected any of this! And the wise juror waits patiently for the counsel for the defence to present his counter-case.

From Newton he will get barely a glimmer of what that might be. Nor is here, in a short review, the place to specify with full subtlety and technicality of argument how time and again he fails to set out fairly the difficulties, theoretical and observational, which Ptolemy had to face in creating his synthesis, imperfect as it was, of existing astronomical knowledge—this through a previous analysis of data, 'truly' reduced from observed phenomena or confected *ad hoc* as they might severally have been, at which we can for the most part make only reasoned conjecture by re-analysing the finished synthesis itself as we alone have it.

The modern fast computer allows us, it is true, freely to apply one power-

ful independent touchstone as to how the Sun, the Moon and the night sky would have appeared to the ancient observer (if we make certain minimal, seemingly well-founded, assumptions about the stability of the 'fixed' stars and the unchanged basic motions, secular and periodic, of the Sun, Moon and planets over the past several thousand years): that of extrapolating back from our modern accepted theories of such apparent motion. And on this test Newton bases the numerous charges of fraudulence in observation which he hurls at Ptolemy. Yet one must be very careful in interpreting divergences between 'observed' values and the predicted past event as it 'must' have been seen.

Modern theories are too exact for the historian's purpose, too well corrected for all manner of disturbing factors to afford a proper basis of comparison. Moesgaard and others have indicated that many of the past recorded sightings (those of lunar eclipses above all, on which depended in classical times all precise geometrical modellings of the Moon's motion) were themselves available to Ptolemy in a version already reduced to norm according to a variety of simple interpretative models about which he had no sure knowledge. And so to some degree it was no sin for him to hone their roughnesses. As for the raw observations themselves, there is no need to lay heavy stress on the gross inaccuracies and imprecisions which beset all sightings by the naked eye using only the most primitive of mechanical aids: at best the Greek astronomers could call on the help of an armillary sphere and a calibrated ring-dial ('astrolabe'), and their only exact means of timing was to use the diurnal motion of the Earth in shooting the Sun and the stars.

All of this Newton takes into account, though without emphasising the size of the errors—15' of arc is nothing—which swiftly agglomerate. There are other less obvious sources of systematic error in taking sightings from our moving Earth which he does not mention at all, such as aberration, and above all atmospheric refraction and—complementary to this as it was measured—the observer's horizontal parallax. (In the late sixteenth century Brahe, the first to give tables for aerial refraction, still set upon the parallax a maximum value of 3', some 20 times too large; and Ptolemy must have made reduction to the Sun by one much greater than this.)

When Mercury was only ever glimpsed near to maximum elongation, around dawn or dusk inevitably when the incoming light ray was bent up to 40' from straight, we ought perhaps to admire Ptolemy for his bravery in

putting his head on the chopping block with any attempted model of the apparent motion of that highly eccentric and perturbed closest satellite of the Sun. And who in honesty can be harsh with him for not more exactly predicting the orbit and changing apparent size of our own Moon when the problem defeated the combined efforts of all—even Newton's seventeenth-century surnamesake (who blandly changed the sign of one inequality in his lunar theory when it suited him)—before Euler and Mayer only two centuries ago?



Claudius Ptolemy

From an engraving by De Brui, about 1648

Not to say that Ptolemy's crank mechanisms for duplicating the motions of both these bodies do not, in their structure as well as their given parameters, rest on readily shakeable foundations. Gingerich, and Delambre long before him, have already shown us some of Ptolemy's eye-deceiving Potemkinist fabrications here. And Newton evinces all the puritanical zeal of the determined iconoclast in endeavouring to prove in the body of his present book that no more depth of reality underlies the rest of the *Syntaxis*; that this "Almagest" is the "greatest" only in the scale and elaborateness of its astronomical *trompe-l'oeil*. In his pages, to be fair, anyone with a passably numerate intelligence and a reasonable degree of historical understanding—this is not a book for the beginner, whatever Newton may say—will find much of the fine web of his explanatory analyses and reconstructions of Ptolemy's mode of thought and composition extremely persuasive, if not everywhere wholly convincing. Again, would there were room here to say more. It makes a good companion to Pedersen's recent exegesis from a different, more conservative viewpoint in his *Survey of the Almagest*, and to the wider pre-

cisions of Neugebauer's *History of Ancient Mathematical Astronomy*. A pity that Newton does not write his controversial account of the *Syntaxis* with a little more of their calmness and humility. One grows weary with the rantings of a pulpit-thumper who interminably moralises and chastises.

In the final count I remain unclear as to exactly what heinous 'crime' Ptolemy here stands charged with. Surely not mere manufacture of evidence in the face of modern canons of scientific propriety, or 'improving' existing data to give a better fit with his preferred structurings of the observable phenomenal reality? Let one look to the working papers of Kepler or Newton (Isaac) in the seventeenth century and see how innocent and amateurish Ptolemy's efforts to round out and confect empirical data were, even if all that is here alleged be true. And if his fabrications, such as they might have been, were indeed a gigantic confidence trick which deceived astronomers for the next millennium and a half and more, is it not that they were knowingly led to believe the myth of his supremacy? Granted that Ptolemy's model of Mercury's orbit makes such a poor fit with what of it can be glimpsed by the naked eye even of an Earth-bound observer, why did the highly competent Arabic astronomers al-Shirazi and his pupil al-Shatir content themselves with so minimal a reshaping of it, one all but exactly preserving Ptolemy's lines of sighting? Yet more astonishing, how was it that Copernicus should in Book 5 of his *De Revolutionibus*, that clarion call to heliocentric 'revolution' as it is so often mistakenly made out to be, timidly settle for parroting al-Shatir's version (as who will now gainsay) of Ptolemy's theory of that planet's motion, one in which the Sun is as ever in motion round the Earth (and cannot be made to stand still in any version of it)? The history of the impact of Ptolemy's *Syntaxis* upon succeeding centuries is a deal more subtle and convoluted than any simple 'Newtonian' view of it as poor theory shabbily propped up by invented observational data, and the whole fraudulently presented to posterity as well-founded celestial science in classical hypothetico-deductive mould can more than start to explain.

If one suspends disbelief at certain passages in it, Newton's book makes an excellent read, but it is only the beginning of a serious estimate of Ptolemy's achievement, frauds and all. □

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articles

Hydration of obsidian

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The hydration of obsidian is studied by measuring hydrogen concentration profiles of natural samples hydrated at 90°C, using the ^{15}N resonance nuclear reaction method. The results of this study are well reproduced by the interdiffusion model of Doremus. The importance of the non-amorphous phase of obsidian is discussed. It is pointed out that the precision methods of measuring hydration profiles now available make it practical to measure hydration rates at room temperature experimentally.

THE study of the reaction between water and obsidian is important to many fields ranging from archaeology, through geochemistry and geophysics, to the technology of radioactive reactor waste consolidation and isolation. Here we investigate the process by which water hydrates a natural obsidian where the hydration is carried out in controlled laboratory conditions. The principal aim is to deal with aspects of the hydration mechanism as it relates to the archaeological technique of dating obsidian artefacts (arrowheads, knife blades, scrapers) based on the extent of hydration on the surface of the object, but the techniques used and conclusions drawn are quite general and should apply to the hydration of many types of glasses.

Understanding the details of the hydration mechanisms is especially important in archaeology to make the best quantitative date assignments based on the hydration¹⁻³. Ideally, the archaeologist would like to be able to measure the extent of hydration on a given artefact and to have a method with which to deduce its rate of hydration. With these data he can assign a date. There are now several methods of varying complexity and accuracy for measuring the extent of hydration, and more methods are likely to be developed^{4,5}. To be able to deduce the hydration rate, we clearly need to begin with an understanding of the hydration mechanism, and this is the aim of this article. Our general conclusion is that the hydration involves the interdiffusion of hydronium ions (H_3O^+) with mobile alkali ions in the glassy phase of the obsidian. The realisation that natural obsidians can have substantial non-amorphous phases is important for understanding some of the results presented here and elsewhere. Although the aim of the present work is to understand the hydration mechanism, it became clear, from measurements made, that the precision methods now available for measuring hydration profiles make it possible to measure hydration rates directly at room temperature.

Experimental

The obsidian samples used in this study were obtained by fracturing a large piece of 'mahogany' obsidian from the western US. The elemental composition of this obsidian is given in Table 1. The fracturing process produced many thin (few mm) pieces of obsidian with large flat fracture surfaces. This method of surface preparation was chosen both because it duplicates that

used to make the archaeological artefacts commonly dated by hydration methods and because our experience with other glasses indicates that other techniques, such as sawing, can affect the apparent hydration rate of the glass. Although no detailed studies are yet available, the sawing may create a network of microcracks which can enhance the initial hydration.

Our experimental procedure is modelled on one developed in a detailed study of the hydration of a commercial soda-lime glass⁶. Our obsidian samples were hydrated for up to 348 h in a bath of temperature-regulated distilled water held at 90°C. The resulting hydration on the surface of the obsidian was profiled quantitatively using the ^{15}N hydrogen profiling method⁷. This uses a narrow isolated resonance in the $^{15}\text{N} + ^1\text{H} \rightarrow ^{12}\text{C} + ^4\text{He} + \gamma$ -ray reaction as a probe for hydrogen and measures the hydrogen concentration in any solid against depth into that solid. This technique has been previously used to measure hydration profiles in soda-lime glass and is fundamentally similar to that used by Lee *et al.*⁸ who were the first to use a nuclear reaction method to profile the surface of hydrated obsidian.

The variable-energy ^{15}N beam from the Yale MP Tandem was used. The experimental arrangement had been calibrated previously, allowing the γ radiation yield curve to be converted to a plot of absolute hydrogen concentration against depth using an energy loss of ^{15}N of $2.0 \text{ MeV } \mu\text{m}^{-1}$. Typical beam intensity was $2 \times 10^{-9} \text{ A}$ with a beam spot of 10 mm^2 . We were concerned that the ^{15}N beam might disturb the hydrogen distribution during the analysis and to check this, some of our measurements were repeated after completing the profile. These repeat measurements showed no change in the hydrogen distribution. Detailed description of the experimental system and a comparison between this method and other analytical techniques for measuring hydrogen profiles are given elsewhere^{7,4}.

Results

The measured hydrogen profiles for our hydrated obsidians are summarised in Figs 1 and 2. Because the rate of hydration is expected to be limited by diffusion, we assumed that the thickness of the hydration profile would increase proportionally to the square root of the hydration time. This is shown in Fig 1 where the measured depth into the sample at which the hydrogen concentration has decreased to half the value near the surface is plotted against the square root of the hydration time: the expected $t^{1/2}$ dependence is obtained.

Clearly, the hydration is proportional to $t^{1/2}$, and so to discuss the shape of the hydration profile, it is convenient to combine all the data by removing this explicit time dependence—that is, to plot hydrogen concentration against depth divided by the square root of the hydration time ($x/t^{1/2}$). This is shown in Fig 2. The typical error bars shown on a few of the points reflect only statistical uncertainties in the data. By thus combining all these data, the statistical uncertainty associated with individual data points as well as any local variations in the composition of the

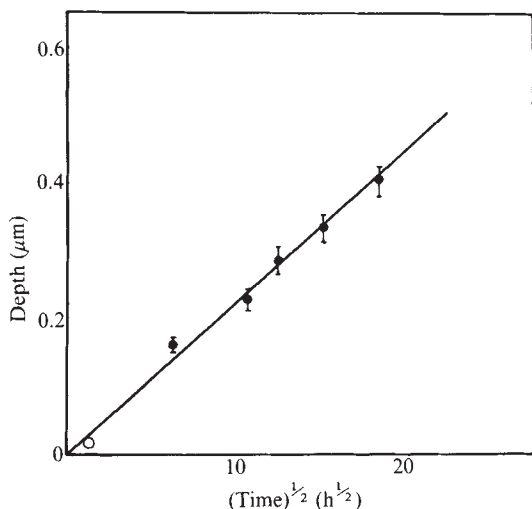


Fig. 1 The penetration depth as function of the square root of the hydration time. The penetration depth is defined as the depth to half-maximum hydrogen concentration. The error bars reflect the statistical uncertainties only. The open circle shows the penetration in an unhydrated sample exposed to air at room temperature for two months. The hydration time has been converted to the equivalent time at 90 °C by an extrapolation according to the Arrhenius equation assuming an activation energy of 20 kcal mol⁻¹.

obsidian become less important as we consider only the average profile.

The hydration profile shown in Fig. 2 has the shape expected from other experimental measurements^{8,5} and for the Doremus interdiffusion model for the hydration of glasses⁹. In fact, the solid lines in Figs 1 and 2 result from a calculation based on this model. It is reassuring that the hydrogen content of the unhydrated obsidian (0.3×10^{21} H atoms cm⁻³) deduced from the ¹⁵N profile measurements agrees with the results of the commercial analysis of the obsidian's composition as given in Table 1.

Hydration mechanism

There has long been interest in the mechanism for the hydration of alkali glasses—such as obsidian—and there are several hypotheses on the important hydration mechanisms. We will compare our results with the Doremus model⁹, in which the hydration is the result of ionic interdiffusion between the alkali ions in the glass with hydronium ions formed at the surface. Doremus compared the shape of the hydration profile predicted by his model with that measured by Lee *et al.*⁸ on the surface of an archaeological artefact. Our more complete results presented here agree entirely with this first single comparison.

A second hydration mechanism which has been commonly mentioned is that of diffusion of molecular water into the glass matrix. We are not aware of reliable experimental evidence favouring this above the ionic interdiffusion model (although molecular water may diffuse into the ion-exchanged hydrated glass). One strong piece of evidence favouring the interdiffusion model is the depletion of alkali ions observed in hydrated glasses, including obsidians. This is observed both in leaching experiments where the amount of ions which diffuse out of the glass is measured, and in direct measurements of the profiles of the alkali elements in the hydrated glass^{5,6}. A second general piece of evidence favouring the interdiffusion model is that the glasses which hydrate are ionic conductors whereas glasses without mobile ions do not generally hydrate (such as SiO₂).

The Doremus model⁹ is based on the fact that in a glass such as obsidian, the only mobile charge carriers are monovalent alkali ions such as Na⁺ and K⁺. These positive ions are very mobile and would diffuse out of the glass were it not for the electrostatic attraction between them and the immobile anions in the glass.

However, if some other positive ion such as hydronium (H₃O⁺) is available to substitute for these alkali ions, the mobile alkali ions and the hydronium ions can interdiffuse, resulting in hydration of the glass. Hydronium ions are available from water on the surface of the glass reacting with Na in the glass: $2\text{H}_2\text{O} + \text{Na}^+(\text{glass}) \rightarrow \text{H}_3\text{O}^+(\text{glass}) + \text{NaOH}$ and through a similar reaction with K⁺. We discuss H₃O⁺ rather than H⁺ or some other ion because results with other glasses indicate that about three hydrogen atoms replace every alkali atom during hydration⁶. The interdiffusion model is convenient because it can be transformed to an equivalent simple diffusion problem with a concentration-dependent diffusion coefficient $\tilde{D} = D_{\text{H}}D_{\text{A}}/(C_{\text{H}}D_{\text{H}} + C_{\text{A}}D_{\text{A}})$ for which standard methods of solution exist where D_{H} and D_{A} are the diffusion coefficients of hydronium and the alkali ion, and C_{H} and C_{A} are their respective concentrations. Hence if one has a glass with a mobile alkali ion (such as Na⁺) interdiffusing with hydronium, one can immediately predict the shape of the hydration profile and the rate of hydration given values of the diffusion coefficients of the Na⁺ and H₃O⁺. In the results presented here, the interdiffusion problem was solved numerically. In principle, when considering the profile shape one also has to consider the etching of the surface of the hydrated obsidian. However, the fact that the data fall in a straight line in Fig. 1 and that obsidians are known to hydrate to thicknesses of many microns implies the etching is very slow, so we have ignored it.

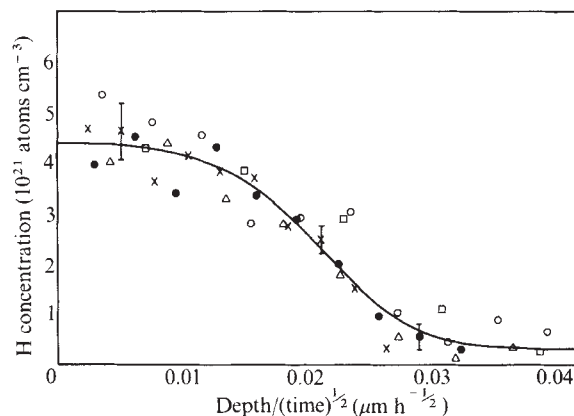


Fig. 2 The measured hydrogen profiles of the hydrated samples are here shown by using a reduced depth scale, which is the actual depth divided by the square root of the hydration time. The solid curve is the prediction of the interdiffusion model with $D_{\text{H}} = 1.4 \times 10^{-16}$ cm² s⁻¹ and $D_{\text{A}} = 1.4 \times 10^{-14}$ cm² s⁻¹. The calculated profile has been normalised to the data in terms of the amount of hydrogen in the fully hydrated glass at the surface (see text). □, 40 h; △, 116 h; ○, 158 h; ●, 234 h; ×, 348 h.

In the present case, the glass contains both Na⁺ and K⁺ (see Table 1), and both ions are expected to interdiffuse with hydronium. However, it turns out that as long as $D_{\text{A}} \gg D_{\text{H}}$ the hydrogen profile predicted by the interdiffusion model is not very sensitive to the precise value of the diffusion coefficient for the alkali ion (D_{A}). Increasing D_{A} by several orders of magnitude has only a small effect on the shape of the hydrogen profile and the rate of hydration. The underlying reason for this insensitivity to D_{A} is that as long as $D_{\text{A}} \gg D_{\text{H}}$ the rate-controlling step in the hydration process is the diffusion of hydronium into the glass.

Hence in a glass, such as the obsidian considered here, one expects the total measured hydrogen profile to represent the sum of two components (one for K and one for Na), but, as both profiles are similar, the sum will not be easily distinguished experimentally from that resulting from a single ion. Hence for simplicity we consider model calculations with only a single alkali ion, but the solution may be interpreted as the sum of two.

Figure 2 (solid line) shows the prediction of the interdiffusion model with $D_{\text{A}} = 1.4 \times 10^{-14}$ cm² s⁻¹ and $D_{\text{H}} = 1.4 \times 10^{-16}$ cm²

s^{-1} . As is evident, this prediction is in excellent agreement with the data. The shape of the predicted profile depends only on the ratio D_A/D_H , whereas the rate (shown in Fig. 1) is only weakly dependent on the ratio and proportional to D_H .

Because there are no measurements of D_A and D_H available, they were treated as free parameters to obtain the agreement between model predictions for the rate of hydration (Fig. 1) and the shape of the profile (Fig. 2). The diffusion coefficient for hydronium, thus obtained, is similar to that needed to understand the hydration of soda-lime glass and is consistent with other reported measurements⁶. The corresponding diffusion coefficient for the alkali ion is one order of magnitude smaller than that needed to understand the hydration of soda-lime glass⁶. However, the present obsidian has much less alkali than the soda-lime glass, which has 15.9% Na_2O , and the D_{Na} decreases rapidly with the concentration of Na^+ ions in the glass. Also, some of the alkali ions are K^+ , which are less mobile than the Na^+ , and hence D_A should be somewhat smaller than D_{Na} . Extrapolating the data for soda-lime glass¹¹ indicates that qualitatively we should expect D_A to be an order of magnitude smaller in this obsidian than in the soda-lime glass of ref. 6, which again agrees with our experimental results. There are also measurements of D_{Na} in obsidian in the temperature range 350–850 °C. Extrapolating these results to the 90 °C of the present hydration implies D_{Na} to be of the order of 5×10^{-14} with an uncertainty resulting from the extrapolation of at least an order of magnitude. Hence we see that the interdiffusion model predicts both the shape and absolute hydration rate with values of D_A and D_H (the only parameters in the theory) consistent with the available measurements of these diffusion coefficients.

One point, not discussed above, is important for reconciling the measurements reported here and their interpretation within the interdiffusion model with the results of others. The hydrogen profile shown in Fig. 2 has been arbitrarily normalised to the data in terms of the amount of hydrogen in the fully hydrated glass at the surface. If the hydrogen-bearing ion which replaces the alkali ions in the glass is hydronium, and if all the alkali ions are allowed to participate in the interdiffusion, then we expect the hydrogen content of the hydrated glass to correspond to a 3-atom hydrogen replacement for each alkali ion, as has been observed in manmade glasses (W.A.L. *et al.* unpublished and ref. 6). The results shown in Fig. 2 indicate only about half this amount of hydrogen. Measurements of the profiles of the alkali ion concentration in hydrated obsidian⁵ also indicate that only about half of these ions are removed during the hydration, in contrast to the results in soda-lime glass where the sodium is completely removed.

The reason the alkali ions are only partially depleted in hydrated obsidian is not completely clear. One possibility is that some of the alkali ions are immobilised and, hence, cannot take part in the ion exchange. This immobilisation may result because in addition to the glassy phase, obsidians typically contain large amounts of crystal inclusions. A recent survey by Ericson *et al.* using optical methods indicated that ~10% of obsidians were crystal inclusions¹³. In addition to the crystal inclusions which can be seen microscopically, X-ray studies of purely 'glassy' obsidian show weakly developed lines which correspond to the minerals to which the obsidian would have crystallised had it cooled more slowly¹⁴. These X-ray studies indicate that obsidians can have small structures which, although not optically visible, may behave more like crystal inclusions than amorphous glass.

A second possibility is that the two alkali ions present (Na^+ and K^+) may interact immobilising some of the ions. This may be related to the dramatic decrease in ionic conductivity observed in mixed alkali glasses over those made with only one alkali element¹⁰.

The presence of a crystal component in obsidian has several implications. The crystal material is expected, if it hydrates at all, to hydrate many orders of magnitude more slowly than the glass. Feldspars contain proportionally much more of the Na and K

Table I Chemical analysis of obsidian

Chemical component	Weight %
SiO ₂	75.85
Al ₂ O ₃	12.81
Fe ₂ O ₃	0.85
FeO	0.57
MnO	0.05
TiO ₂	0.25
CaO	0.48
MgO	0.16
Na ₂ O	3.74
K ₂ O	4.20
H ₂ O ⁺	0.15
H ₂ O ⁻	0.008
P ₂ O ₅	0.03
CO ₂	0.05
Total	99.148

The analysis was carried out by York Research Corporation, Stamford, Connecticut, using atomic absorption spectroscopy and other methods.

than the bulk composition and, consequently, their existence substantially depletes the alkali component of the glass phase. A 10% component of potassium or sodium feldspar incorporates ~20% of the total alkali. In the present case, it is difficult to estimate quantitatively how much of the sodium and potassium given in the bulk composition (Table I) exists in the glassy phase and should then exchange with hydrogen in the hydration process: if 10% of the obsidian is crystallised in potassium or sodium feldspars¹³ and if roughly an equal amount of alkali is immobilised in embryonic crystals, then the observed hydrogen content is consistent with each alkali ion being replaced with one H_3O^+ ion.

The suggestion that a fraction of the alkali ions in obsidian is immobilised is consistent not only with this interpretation of our present data but also with the recently reported⁵ partial depletion of alkali ions in hydrated obsidian. It also provides a natural explanation for the fact that no correlation was found between alkali content and hydration rate for a variety of obsidians¹, whereas it is well known that for synthetic glasses the alkali content is key to the hydration¹⁰. If an unknown fraction of the alkali is immobilised in crystalline structures, the bulk alkali content may not reflect the true alkali content in the glass phase of the obsidian. On the other hand, the interdiffusion model predicts an increase in hydration rate with alkali content because of the known increase in alkali diffusion rate with increased alkali content. This should also be realised as a correlation between the maximum hydrogen content in fully hydrated obsidian with hydration rate. Such a correlation has already been reported in the hydrogen profile measurements of Lee *et al.*⁸ The importance of the non-amorphous phase of obsidian in explaining the lack of correlations between bulk composition and density has already been reported¹³.

Direct measurement of hydration rate at room temperature

The ¹⁵N hydrogen profiling method can quantitatively measure hydrogen profiles as thin as a few hundred Angstroms. If the thickness of the hydrated surface layer (X) varies with time as $X^2 = KT$, archaeological evidence shows K varies from <1 to 20 μm^2 per 1,000 yr (ref. 2). Assuming, for example, $K = 10 \mu m^2$ per 1,000 yr means that, if a piece of obsidian is fractured and the resulting fresh fracture surface is exposed to the ambient environment for one year, the resulting hydration layer will be 1,000 Å thick. This is easily measurable by the ¹⁵N hydrogen profiling method. By such a procedure, it should be possible to determine the hydration rate for individual artefacts.

For example, the open data point in Fig. 1 is the hydration thickness (200 Å) of a sample of obsidian which was not exposed

to the 90 °C water bath but which, instead, was in an air environment for two months before being profiled. The hydration rate deduced corresponds to $K = 2.4 \mu\text{m}^2$ per 1,000 yr, which agrees with that predicted from the archaeological results.

A practical use of such measurements might be to fracture the artefact to be dated and return it to the environment in which it was found for one year; by measuring the hydration profile on the 'one-year-old' surface, a hydration rate is determined which is automatically averaged over annual temperature variations. By then measuring the hydration profile on an original surface, the age of the artefact could be deduced.

Conclusion

We see that the interdiffusion model predicts the shape of the hydration profile and the rate of hydration in good agreement with our data. The values of the two free parameters in this model (D_A and D_H) which are needed for agreement are, as well as can be presently determined, consistent with other measurements. Finally, we emphasise that the experimental

methods described here can be useful in characterising the hydration rates of other glasses, whether the glass is a single archaeological artefact^{2,15}, a scientific or commercial glass, or a glass designed to isolate radioactive reactor wastes.

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1. Friedman, I. & Long, W. *Science* **9**, 347 (1976).
2. Friedman, I. & Trembour, F. W. *Am. Scient.* **66**, 44 (1978).
3. Taylor, R. E. (ed.) *Advances in Obsidian Glass Studies* Noyes Press, Park Ridge, New Jersey, 1976.
4. Ziegler, J. F., et al. *Nucl. Instrum. Meth.* **149**, 19 (1978).
5. Tsong, I. S. T. et al. *Science* **201**, 339 (1978).
6. Lanford, W. A. *Nucl. Instrum. Meth.* **149**, 1 (1978).
7. Lanford, W. A., Trautvetter, H. P., Ziegler, J. & Keller, J. *Appl. Phys. Lett.* **28**, 566 (1976).
8. Lee, R. R., Leich, D. A., Tombrello, T. A., Ericson, J. E. & Friedman, I. *Nature* **250**, 44 (1974).
9. Doremus, R. H. *J. Non-Crystal. Solids* **19**, 137 (1975).
10. Doremus, R. H. *Glass Science* (Wiley, New York, 1973).
11. Terai, R. & Hayami, R. *J. Non-Crystal. Solids* **18**, 217 (1975).
12. Carron, Jean-Paul C. R. *hebd Seanc. Acad. Sci. Paris Ser. D* **854** (1968).
13. Ericson, J. E., Makishima, A., MacKenzie, J. D. & Berger, R. *J. Non-Crystal. Solids* **17**, 129 (1975).
14. Ellis, A. J. & Mahon, W. A. *J. Geochim. cosmochim. Acta* **31**, 519 (1967).
15. Lanford, W. A. *Science* **196**, 975 (1977).

Oceanic plate motions driven by lithospheric thickening and subducted slabs

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The thickening of the cooling oceanic lithosphere results in a significant driving force that is distributed over the area of the plates. Simple flow models show that this force may be more important in driving oceanic plates than that force from sinking lithospheric slabs at subduction zones.

IN the past decade, the theory of plate tectonics has been quite successful in explaining many geological and geophysical observations. Nevertheless, plate tectonics remains primarily a kinematic theory. Although some form of thermal convection must be responsible for the plate motions¹, the details of the driving mechanism and flow in the mantle accompanying the plate motions are still uncertain. The lithosphere, the mechanically strong thermal boundary layer which makes up the plates, probably has an important influence on mantle flow: the largest temperature gradients in the Earth occur across the lithosphere. As convection is driven by the body forces caused by differences in temperature, the plates might be expected to play an important part in governing convection in the Earth. Thus, to understand the dynamics of mantle convection, it is important to isolate and understand the effects resulting from the plates themselves.

Because the plates are denser than the mantle below, they are gravitationally unstable and ultimately sink into the mantle at subduction zones. The sinking of the dense subducted slabs into the mantle provides a potentially important driving mechanism for plate motions which has been considered in several investigations of the forces driving the plates¹⁻⁴.

Important density contrasts are not, however, limited to the sinking slabs. As the oceanic lithosphere moves away from the ridge where it is created, it becomes thicker because cooling penetrates deeper into the mantle. This thickening of the dense lithosphere leads to horizontal density differences across distances of the order of the plate dimensions. Simple flow models presented here show that these density contrasts are sufficient to drive plate motions at rates of centimetres per year. In fact, the

body forces from the thickening of the lithosphere seem to be even more important in driving plates than the body forces associated with the subducted slabs.

Model of the lithosphere

The thermal evolution of the oceanic lithosphere is fairly well understood. Many of the properties of the oceanic lithosphere can be explained by simple models based on cooling the mantle from above^{1,5-8}. The essential features of these models are illustrated by a model of a cooling halfspace^{6,7}. In this model, shown in Fig. 1, mantle material at temperature T_0 rises in the vicinity of an oceanic ridge and moves horizontally away from the ridge at a velocity u which is constant and independent of depth. At distances not too close to the ridge, the temperature at depth z is given in terms of an error function:

$$T(z) = T_0 \operatorname{erf}(\zeta/2(\kappa t)^{1/2}) = T_0 \operatorname{erf}(\zeta/2(\kappa x/u)^{1/2})$$

where κ is the thermal diffusivity, t is the time since the material was at the ridge, and x is the horizontal distance from the ridge. The second equality results from the relationship between time and distance from the ridge, assuming constant spreading velocity u .

If the base of the thermal boundary layer constituting the lithosphere is defined by some critical temperature T_i , the thickness of the lithosphere d is given by

$$d = 2(\kappa x/u)^{1/2} \operatorname{erf}^{-1}(T_i/T_0)$$

It is a property of the error function solution that the average temperature, and therefore the average density, of the lithosphere is independent of distance from the ridge. Thus the lithosphere can be thought of as a cool, dense, mechanically strong layer of constant mean density which grows thicker away from the ridge as the material formerly below the lithosphere cools and becomes part of it.

As the dense lithosphere thickens by accretion, it subsides into the less dense mantle beneath, resulting in an increase in ocean depth away from the spreading ridge. This sinking is

inferred to lead to an essentially complete isostatic compensation of the thickening lithosphere because the free air gravity anomalies observed over oceanic ridges and basins are small⁹. Isostasy requires that the elevation of the seafloor, η , and the elevation of the base of the lithosphere, η_b , both referred to their final depths just before subduction, are related by

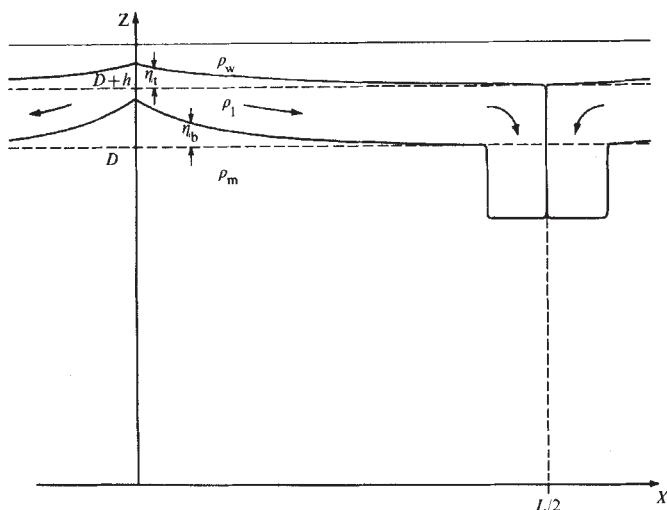
$$\eta_b = ((\rho_l - \rho_w)/(\rho_l - \rho_m))\eta_t$$

where ρ_l , ρ_w , and ρ_m are the densities of the lithosphere, seawater, and the mantle, respectively. The boundary layer theory predicts that the increase in depth of the ocean owing to the isostatically compensated thermal contraction of the surface layer should depend on the square root of age or distance from the ridge crest, in accord with observation to plate ages of at least 80 Myr (ref. 8).

Eventually, the dense plate plunges into the mantle at a subduction zone. As it sinks it may pull the rest of the plate with it, as suggested by Elsasser¹⁰. Simple dynamic models of mantle flow⁴ and phenomenological studies of forces on plates^{2,3} have indicated that the body forces of the sinking slab may provide an important part of the forces driving the plates, although detailed models involving the transmission of the force from the sinking slab to the horizontal plate have not yet been analysed.

The thickening of the lithosphere itself is another source of density contrast that can cause flow and plate motion. Figure 1 shows that the dense lithosphere is separated from the less dense mantle below by a sloping interface. The situation is analogous to a dense fluid overlying a less dense fluid in a tank. Clearly, if the interface between the two fluids slopes, the configuration is not in equilibrium, and the dense fluid will begin to sink at its thick end while the less dense fluid will rise at the other end where the dense layer is thinnest. Similarly, the thick lithosphere far from the ridge will sink, and the less dense mantle will rise under the ridge. There will be an accompanying surface flow from ridge to trench, corresponding to plate motion, with a return flow at depth to complete the mass circuit. As the mantle material rises continuously under the ridge, it cools and accretes onto the dense lithosphere. The lithosphere is reheated and resorbed as it sinks into the mantle; this would cause steady state thermal convection driven by body forces associated with the thickening of the lithosphere at the surface if accretion and resorption occurred at the appropriate rate.

Fig. 1 Schematic representation of the geometry assumed in the flow models. The elevation of the sea floor, η_i , and the elevation of the base of the lithosphere, η_b , are related because the lithosphere is in isostatic equilibrium. Because the density of the mantle, ρ_m , is less than the density of the lithosphere, ρ_l , horizontal density contrasts due to the thickening lithosphere and subducted slab drive the plate towards the trench. The core-mantle boundary is at $z = 0$.



The velocity of this plate-driven flow depends on the extent of the horizontal density differences. If the top and bottom of the lithosphere were flat, there would be no horizontal density differences, so there would be no motion. One measure of the magnitude of the driving force is the horizontal mass difference, the volume integral of the horizontal density contrast. If isostatic compensation is assumed, the horizontal mass difference accompanying the change in depth of the base of the lithosphere is equal in magnitude to the horizontal mass difference accompanying the change in elevation of the sea floor. The total horizontal mass difference is $9.2 \times 10^{12} \text{ kg m}^{-1}$ for a ridge whose elevation decreases as the square root of the distance, from 3 km at the ridge crest to 0 km, 6,000 km away assuming a density difference of 2.3 Mg m^{-3} between the lithosphere and seawater.

The total horizontal mass difference inherent in the subducted slab is not as well constrained as that resulting from the thickening of the cooling plate because the thermal evolution of a subducted slab depends on complicating factors such as viscous dissipation, phase changes, and the variation of the ambient temperature of the mantle with depth¹¹. In a model of the evolution of a 120-km thick slab calculated by Schubert *et al.*¹², including the effects of frictional heating and phase transitions, the horizontal mass difference is $4.5 \times 10^{12} \text{ kg m}^{-1}$, about a third of which results from elevation of the phase boundary between the olivine and spinel phases at a depth of 350 km. This model predicts a much larger horizontal mass difference than other slab models^{1,11} but this mass difference is still a factor of 2 smaller than that calculated for the thickening of the cooling surface plate. Thus the body force associated with the cooling plate may be more important in driving plate motions and mantle flow than the body forces associated with the sinking slabs. Of course, the flow in the mantle owing to a distribution of body forces depends on the position as well as the magnitude of the body forces, so it is necessary to compute flow models before evaluating the relative importance of these two body forces resulting from the thermal evolution of the lithosphere.

Viscous flow models

To facilitate comparison of the relative magnitudes of the two driving mechanisms, the flow driven by the thickening lithosphere alone is computed for comparison with the flow driven solely by the sinking slab. The rheology of the mantle is taken as newtonian viscous and the fluid is incompressible, which is consistent with the commonly used Boussinesq approximation. Neither assumption is strictly justified for flow in the Earth, but this is not expected to invalidate the qualitative conclusions to be drawn from the models. Plate geometries and the distribution of body forces are assumed to be in steady state, so there is no time dependence in the models; streamlines and particle paths coincide.

The Stokes equations for a fluid in a two-dimensional Cartesian frame are solved by decomposing horizontal variations of the flow variables into Fourier series and solving for their depth dependences for each term in the series. Where volumetric density variations exist, they are expressed as equivalent surface density distributions on planes spaced closely enough that the computed flow pattern is not affected appreciably by this approximation.

The shear stress is zero at the top and bottom of the model, which correspond to the ocean floor and core-mantle boundary. According to standard practice^{6,19}, no vertical displacement of these boundaries is permitted. The vertical displacement which would result if a boundary were permitted to deform is, to first order, the topography which could be supported by the normal stress at the undeformed boundary. As this topography is negligible compared to the depth of the model, the flow geometry is not appreciably affected by this approximation. The boundary conditions at the sides are also free slip, equivalent to the periodic array of plates shown in Fig. 1. The width of each cell is 5,570 km. The models shown below have a viscosity of 10^{21} N s m^{-2} throughout, the average mantle viscosity determined in studies of postglacial rebound¹³.

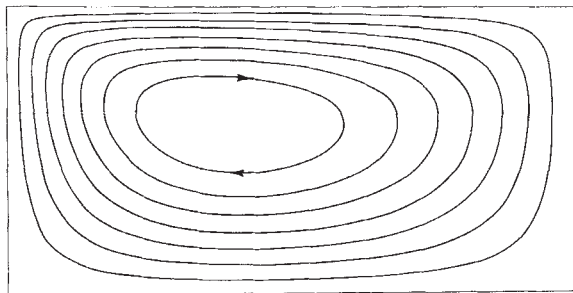


Fig. 2 Streamlines for flow driven only by the thickening lithosphere. The maximum magnitude of the stream function is $8.2 \times 10^7 \text{ cm}^2 \text{ yr}^{-1}$ and the contours are equally spaced. The average surface velocity is 1 cm yr^{-1} . The maximum velocity is 1.6 cm yr^{-1} and occurs at the surface. The viscosity is $10^{21} \text{ N s m}^{-2}$ throughout.

The streamlines for flow driven by the thickening lithosphere are shown in Fig. 2. The horizontal density contrast from the thickening of the plate is included at a depth of 64 km. The density contrast is that needed to compensate for a seafloor elevation decreasing as the square root of the distance from the ridge from a maximum elevation of 3.0 km, the topography appropriate for a spreading rate of 7 cm yr^{-1} . A density contrast between the lithosphere and seawater of 2.3 Mg m^{-3} is assumed.

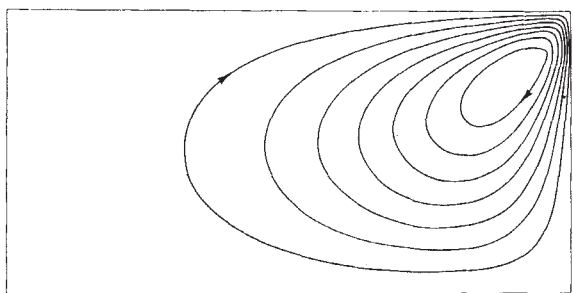
The average velocity at the surface is 1 cm yr^{-1} . Near the surface, the streamlines are essentially parallel, indicating that the flow driven by the thickening lithosphere causes little deformation of the surface. Calculated shear stresses are of the order of 0.5 MPa.

Details of the flow depend on the viscosity structure. Increasing the viscosity in the lithospheric layer by a factor of 10 decreases the average surface velocity by a factor of 2. The average surface velocity increases to 16 cm yr^{-1} if a 64-km thick low viscosity channel of $10^{18} \text{ N s m}^{-2}$, similar to that proposed to lie under oceans by Artyushkov¹⁴, is included at the base of the lithosphere. One could easily match the surface velocity of 7 cm yr^{-1} with the density distribution driving the flow in this model by a suitable selection of parameters and hence the model of the plate-driven flow can be made entirely self-consistent.

The streamlines for flow driven by the sinking of a subducted slab are shown in Fig. 3. The density contrast in the slab decreases linearly with depth from 0.06 Mg m^{-3} at the surface to zero at a depth of 670 km. This decrease in density with depth, which might result from heating the slab as it sinks, was chosen so that the calculated stresses would roughly match the seismic observation that slabs are in down-dip compression below a depth of 300 km (ref. 15).

The average velocity at the surface is 1 cm yr^{-1} , although the velocity reaches 8 cm yr^{-1} in the region of the sinking slab, where the flow is concentrated. The converging streamlines along the

Fig. 3 Streamlines for flow driven by a sinking slab. The density contrast in the slab varies linearly from 0.06 Mg m^{-3} at the surface to 0 Mg m^{-3} at a depth of 670 km. The maximum amplitude of the stream function is $14.8 \times 10^7 \text{ cm}^2 \text{ yr}^{-1}$ and the contours are equally spaced. The average surface velocity is 1 cm yr^{-1} , although vertical velocities in the subducted slab reach 8 cm yr^{-1} . The viscosity is $10^{21} \text{ N s m}^{-2}$ throughout.



surface indicate that the near-surface layers experience extensional stress.

As before, varying the viscosity structure changes the flow somewhat. Increasing the viscosity of the lithosphere by an order of magnitude decreases the average surface velocity by a factor of 2. Alternatively, including the 64 km thick low viscosity channel of $10^{18} \text{ N s m}^{-2}$ leads to an average surface velocity of 6 cm yr^{-1} , compared with 16 cm yr^{-1} for the same viscosity distribution with the flow driven by the thickening lithosphere.

Discussion

For the constant viscosity models, the average surface flow driven by the thickening lithosphere is as large as that driven by the sinking slab. A low viscosity channel increases the surface flow for the thickening lithosphere model more than for the slab model. Also, the 670-km slab in the model is longer than the seismically determined length of most subducted slabs¹⁵. Therefore, on a worldwide basis, the surface flow driven by the thickening oceanic plates might well be larger than the surface flow driven by subducted slabs.

For viscous flow driven by body forces, the scale of the flow depends on the distribution of those forces. The density differences associated with thickening oceanic plates are spread out over distances equal to the dimensions of the plates, while the body forces associated with subducted slabs are more localised. The flow driven by the sinking slabs is concentrated in the region of subduction, where it may be important in the local deformation associated with bending and underthrusting of the lithosphere. In contrast, the flow driven by plate thickening is more global in scale, in accord with plate motions that are coherent over large distances.

The predicted dependence of ocean depth on the square root of age is not valid beyond about 80 Myr, which corresponds to lithospheric thickness on the order of 130 km, after which the thickness may not increase appreciably⁸. Consequently the driving force from the thickening lithosphere is distributed primarily over that part of a plate which is <80 Myr old. For very slowly spreading plates, this force can probably be approximated as a boundary force acting on the ridge itself^{1-4,14,16}. Nevertheless, as Hales¹⁷ and Lister¹⁸ have pointed out, for spreading rates typical of oceanic plates, this driving force will be distributed over substantial areas of the plates.

The distinction between an edge force and a distributed force has important dynamic implications. The velocities of oceanic plates are observed to be relatively independent of area; the small Cocos Plate, the intermediate Nazca Plate, and the large Pacific Plate all move at about the same rate². For plates spreading at equal rates, the driving force from the thickening lithosphere is greater for large plates than for small ones. Also, the body force in the subducted slab, which must depend to a large extent on the time a plate cooled at the surface before subducting, should be greater for a large, old plate than for a small, young one. For the plate velocities to be relatively independent of area, there must be a resisting force which increases as the area of a plate increases. Drag at the base of the lithosphere is likely to satisfy this criterion.

For drag at the base of the lithosphere to be an important resisting force, oceanic plates, on average, must be moving faster than the underlying mantle. Richter showed that the shear generated by a moving lithosphere should exert a strong organising influence on the flow in the mantle, resulting in a coherent large scale convection pattern dominated by the plate motions¹⁹.

The nature of the force resulting from the thickening of the oceanic lithosphere suggests that oceanic plate velocities might be self-limiting. The slope at the base of the lithosphere is greater for slowly moving plates than for rapidly moving ones. Therefore, the driving force from the thickening of the plates is larger for slowly moving plates, all other factors being equal. If a plate were to move away from a ridge more rapidly, the ridge topography and accompanying topography at the base of the

lithosphere would become flatter, providing less drive. Oceanic plates might all move at about the same speed² because their associated ridges are spreading at terminal velocity, rather than because their slabs are sinking at terminal velocity²⁻⁴, as old slabs might be expected to have a larger terminal velocity than young ones.

Conclusions

The density contrast at the Earth's surface associated with the cooling and thickening of the oceanic lithosphere provides a significant driving force for plate motions. Simple numerical models of mantle flow show that this plate-driven flow may be more important in generating coherent surface motions than the sinking slab, although flow driven by the sinking slab is likely to be important locally in the region of subduction. Although these models do not consider how oceanic plate motions originate, they do show that the horizontal density differences resulting from the cooling of these plates are capable of sustaining this motion.

The driving force on a plate from the thickening oceanic lithosphere is distributed over the area of the plate. As oceanic plate velocities do not depend on plate area, there is likely to be a significant resisting drag proportional to area at the base of the lithosphere. Thus the plate motions should be important in determining the large scale flow in the mantle.

The magnitude of the plate-driven flow depends on the slope of the boundary between the dense lithosphere and the underlying mantle. As this slope decreases as spreading rate increases, plate-driven flow may be self-limiting, helping to account for the observation that oceanic plate velocities show little variation.

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1. McKenzie, D. P. in *The Nature of the Solid Earth* (ed. Robertson, E. C.) 323-360 (McGraw-Hill, New York, 1972).
2. Forsyth, D. & Uyeda, S. *Geophys. J. R. astr. Soc.* **40**, 163-200 (1975).
3. Chapple, W. M. & Tullis, T. E. *J. geophys. Res.* **82**, 1967-1984 (1977).
4. Richter, F. M. *Tectonophysics* **38**, 61-88 (1977).
5. Langseth, M. G. Jr, Le Pichon, X. & Ewing, M. J. *geophys. Res.* **71**, 5321-5355 (1966).
6. Turcotte, D. L. & Oxburgh, E. R. *J. Fluid Mech.* **28**, 29-42 (1967).
7. Davis, E. E. & Lister, C. R. B. *Earth planet. Sci. Lett.* **21**, 405-413 (1974).
8. Parsons, B. & Sclater, J. G. *J. geophys. Res.* **82**, 803-827 (1977).
9. Kaula, W. M. in *The Nature of the Solid Earth* (ed. Robertson, E. C.) 385-405 (McGraw-Hill, New York, 1972).
10. Elsasser, W. M. in *The Application of Modern Physics to the Earth and Planetary Interiors* (ed. Runcorn, S. K.) 223-245 (Wiley-Interscience, London, 1969).
11. Minear, J. W. & Toksöz, M. N. *J. geophys. Res.* **75**, 1397-1419 (1970).
12. Schubert, G., Yuen, D. A. & Turcotte, D. L. *Geophys. J. R. astr. Soc.* **42**, 705-735 (1975).
13. Cathles, L. M. *The Viscosity of the Earth's Mantle* (Princeton University Press, 1975).
14. Artyushkov, E. V. *J. geophys. Res.* **78**, 7675-7708 (1973).
15. Isacks, B. & Molnar, P. *Rev. Geophys. Space Phys.* **9**, 103-174 (1971).
16. Jacoby, W. R. *J. geophys. Res.* **75**, 5671-5680 (1970).
17. Hales, A. *Earth planet. Sci. Lett.* **6**, 31-34 (1969).
18. Lister, C. R. B. *Nature* **257**, 663-665 (1975).
19. Richter, F. M. *Rev. Geophys. Space Phys.* **11**, 223-287 (1973).

Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes

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The bactericidal action of colicins K, E1, Ia, and other functionally related colicins involves disruption of active transport and leakage of ions from the cell. We show that a single colicin K molecule can form a voltage-dependent, relatively nonselective, ion-permeable channel of a few picosiemens conductance in a planar phospholipid bilayer membrane. In a membrane containing many of these channels, the ratio of the number of conducting to nonconducting channels changes e-fold per 3.7 mV. We suggest that the physiological effects of colicin K and functionally related colicins result from their ability to form ion-permeable channels in the bacterial plasma membrane.

COLICINS, bactericidal proteins (MW 50,000-100,000) produced by *Escherichia coli*, are of three major functional types: E1, E2 and E3. (For reviews of the physiology of colicin action see refs 1 and 2.) The cellular targets of action of types E2 and E3 are DNA and ribosomal RNA, respectively. The precise target and mechanism of action of the E1 type are not known, but these colicins are thought to act by making ion-permeable channels in the bacterial plasma membrane because: (1) they discharge the 'energised state' of the plasma membrane¹⁻⁷ without inhibiting respiration^{1,8,9} or inducing measurable proton flux^{6,7,10,11}, and (2), an early effect of their action is net efflux of potassium from the bacterium^{10,12-17}. (As macroscopic electroneutrality requires that net potassium efflux be accompanied by either efflux of anions and/or influx of other cations, the

colicin-induced permeability is apparently rather nonselective.) Channel formation by these colicins is also consistent with observed single hit killing¹⁸⁻²⁰, and a pH or voltage dependence of the induced channels would explain the protective action of uncouplers of oxidative phosphorylation (such as FCCP) and of other treatments that discharge the energised state of the plasma membrane^{16,18,21}.

We report here that colicins K, E1, and Ia (members of the E1 functional type) form voltage-dependent, relatively nonselective ion-permeable channels in planar phospholipid bilayer membranes. We believe that this is their primary action on cells and accounts for the observations cited above. We present here data obtained from colicin K; closely related effects are obtained with E1 and Ia.

Conductance is a function of voltage

Planar phospholipid bilayer membranes (area $\approx 0.01 \text{ mm}^2$) separating two salt solutions were formed at room temperature by the union of two monolayers²² of crude soybean phospholipid (lecithin type II from Sigma)²³ from which neutral lipid was removed²⁴. After addition of colicin K to the front (or *cis*) compartment to a concentration of from 0.2 to 200 ng ml⁻¹, known voltages were applied across the membrane, and the resulting current responses were measured²⁵. The conductance (g) of the membrane in symmetric salt solutions is defined as current divided by voltage ($g \equiv I/V$), where V is the potential of the rear (or *trans*) compartment; in the absence of colicin, g is about 10 pS.

Figure 1 shows the typical response of a membrane which separates symmetric salt solutions and has been treated with

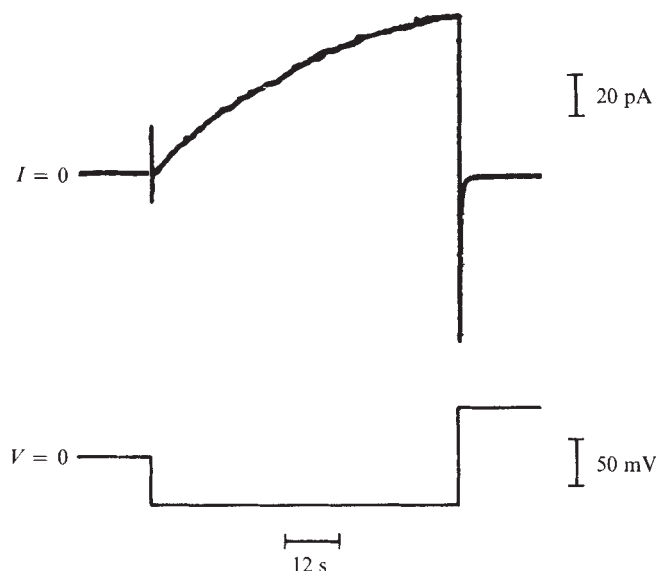


Fig. 1 Current response of colicin K-treated membrane to voltage. After formation of the membrane (in 100 mM KCl + 5 mM CaCl_2 + 1 μM EDTA + 5 mM 2[N-morpholino]ethane sulphonic acid, all adjusted to pH 6.1 with KOH), colicin K produced by *E. coli* was added to the front (or *cis*) compartment to a concentration of 100 ng ml⁻¹, and the record was obtained about 10 min later. The *cis* compartment was connected to virtual ground; *V* is the potential of the rear (or *trans*) compartment, analogous to the inside of the target bacterium. At the 'blip', a voltage of -50 mV was applied across the membrane. The current rises with approximately $1 - \exp(-t/\tau)$ kinetics ($\tau \approx 25$ s) to a steady-state value of 80 pA, which corresponds to a conductance of 1,600 pS. At this point the potential was reversed to +50 mV. The instantaneous current is of the same magnitude but opposite sign as that at -50 mV, and decays rapidly to a very low value. The steady-state colicin-induced conductance at -50 mV is 400 times that observed at +50 mV. Stock solutions of colicin (~ 1 mg ml⁻¹) in 500 mM KCl + 50 mM potassium phosphate (pH 6.0) or in 300 mM NaCl + 50 mM Tris (pH 7.4) were stored in small vials at -70 °C. For a given day's experiments, the contents of a vial were thawed and, along with subsequent dilutions, stored on ice. Colicin K handled in this way was indefinitely stable (>6 months) with respect to its action on lipid bilayers; when left at room temperature for a day or two, however, it lost virtually all of its activity on bilayers.

colicin K on one side. When a negative voltage of sufficient magnitude (in this case -50 mV) is applied, current (and therefore conductance) rises with approximately $[1 - \exp(-t/\tau)]$ kinetics, with τ in this case about 25 s and the steady-state conductance about 1,600 pS. If the sign of the voltage is reversed after this rise, the current initially has the same magnitude (but, of course, the opposite sign), and then decays rapidly within a second to a low value little greater than that for an unmodified membrane. The steady-state conductance is extraordinarily voltage dependent (Fig. 2). Conductance is maximal at large negative membrane potentials and is virtually zero at potentials > -10 mV (Fig. 2a). τ is also voltage dependent, decreasing as $|V - V_0|$ increases. (See below for definition of V_0 .) Over long periods ($t \gg \tau$), there is a slow linear rise in the magnitude of the voltage-dependent conductance.

Single channel behaviour

The colicin-induced conductance described above is due to ion-permeable channels. At small colicin concentrations, discrete current 'jumps' are seen at constant voltage (Fig. 3). The conductances corresponding to these jumps are fairly uniform and, in 100 mM KCl, are about 1.5–3 pS for colicin K produced by *E. coli*. For colicin K produced by *Proteus mirabilis*, the jumps are about 10 pS; this surprising difference is discussed below. These unit conductance jumps are much too large for a shuttling

ion carrier, and must therefore result from ion-permeable channels^{26,27}; they are comparable in size to those of the sodium and potassium channels in nerve and the cholinergic channels at the neuromuscular junction²⁸. The linear dependence of maximal steady-state conductance on colicin concentration (Fig. 4) implies that each voltage-dependent channel is formed by a single colicin K molecule.

The macroscopic voltage dependence (Fig. 2) is reflected in single channel behaviour (Fig. 3); that is, channels are almost always on at large negative voltages and almost always off at small negative or positive voltages. The data in Fig. 2a are replotted in Fig. 2b according to the Boltzmann relationship²⁵. Over a wide voltage range the ratio of the number of open to closed channels changes *e*-fold per 3.7 mV. The potential at which half the channels are open (ratio = 1) defines the 'switching voltage' (V_0) of the channel, in this case -31 mV.

Similar results were obtained in membranes formed from whole *E. coli* phospholipids or from mixtures of purified bacterial phospholipids mimicking that composition. The number of channels obtained for a given concentration of colicin was 1–10% of the number obtained with membranes formed from crude soybean phospholipids.

Ion selectivity

Colicin K-induced channels are cation selective, but poorly so. A ratio of 10:1 for NaCl concentrations across the membrane (50 mM:500 mM) produces a diffusion potential of 33 mV (dilute NaCl side positive). This was established by determining the reversal potential either for the macroscopic current produced by many channels or for the individual current produced by a single channel (Fig. 5). This potential implies a selectivity of Na^+ to Cl^- of about 4:1. Essentially the same result is obtained with gradients of KCl, indicating little discrimination by the channel between K^+ and Na^+ ; the absence of a significant bi-ionic potential for KCl/NaCl confirms this. The channel also admits divalent cations; in a fivefold gradient of CaCl_2 (20 mM:100 mM) the diffusion potential is approximately 2.5 mV (dilute side negative), indicating a slight preference for Ca^{2+} over Cl^- .

The colicin molecule itself forms the channel

Addition of colicin K preparations to one side of planar phospholipid bilayer membranes induces the formation of strongly voltage-dependent, relatively nonselective channels. It is necessary to establish that these channels are not produced by a contaminant in the preparations, rather than by the colicin itself, because the actual number of channels induced in the membrane represents a small fraction of the number of molecules added to the aqueous phase. [This low efficiency is not surprising; it is generally seen with channel-forming molecules (such as gramicidin A) added to the aqueous phase, and probably results from slow kinetics of insertion into the membrane as well as nonspecific adsorption to other surfaces.]

We think it is very unlikely that a contaminant produces these channels. First, these channels are obtained from two separate preparations of colicin K, including a very pure sample from *P. mirabilis*. Second, we have found that colicins E1 and Ia, which act very much like colicin K on cells^{1,2,17}, also create voltage-dependent, ion-permeable channels in planar phospholipid bilayer membranes. (Whereas the behaviour of colicin Ia is qualitatively similar to that of colicin K, preliminary experiments indicate that the details of the voltage-dependent step(s) in colicin E1 action may be somewhat different.) In contrast, colicins E2 and E3, whose known targets are DNA and rRNA, respectively^{1,2}, are without effect on the bilayers—with the following caveat. At colicin E2 concentrations 1,000 times larger than those used in colicin K, E1 or Ia experiments, we obtained comparable channel activity. We suspect that this resulted from a small amount of Ia contamination of the E2 preparation; this is not unexpected, as the bacterial source of E2 also carried the Ia gene (T. Boon, personal communication). Furthermore, the single-channel conductance in 100 mM KCl

(~7 pS) and the kinetics of the 'E2 contaminant' were indistinguishable from those of Ia. Also, J. Konisky (personal communication) has found that E2 has a small amount of Ia-like activity which is neutralised by anti-Ia antibodies. The permeability effects observed with E2 on cells²⁹ may result from this same contamination. Third, two colicins K, produced by supposedly identical plasmids in *E. coli* and *P. mirabilis*^{30,31}, differ in their single channel conductances (see above). Two independent laboratories have since found that these two colicins differ in molecular weight as determined by SDS-polyacrylamide gel electrophoresis. Colicin K prepared from *E. coli* has a molecular weight of about 5,000 less than that of colicin K prepared from *P. mirabilis* (S. E. Luria and C. Kayalar; K. Jakes, personal communications). Thus, there is a correlated difference in artificial membrane behaviour and biochemistry.

These findings leave little doubt that the channel activity described in this article is produced by colicin K.

Channel activity explains cellular pathophysiology

We believe that the ability of colicin K to make channels in lipid bilayer membranes accounts for the salient features of its action on bacteria as summarised below. In considering this action, we

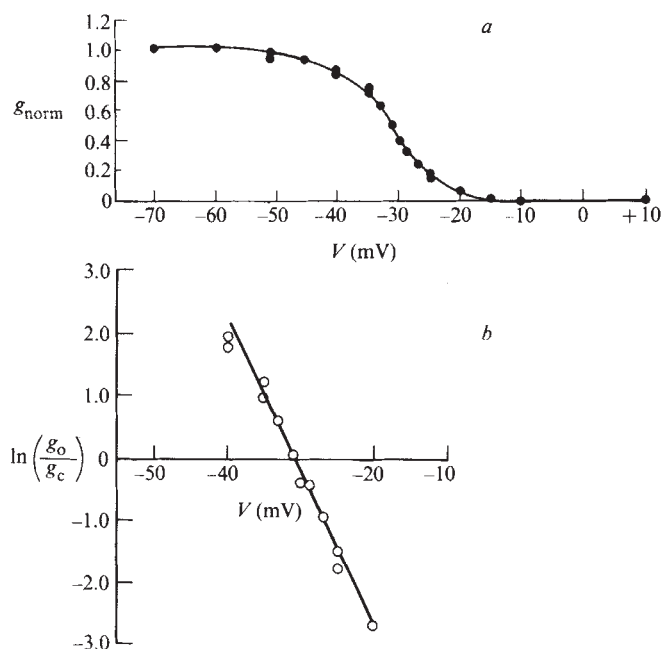


Fig. 2 Voltage dependence of the colicin K-induced conductance. *a*, Normalised steady-state, colicin K-induced conductance (g_{norm}) plotted as a function of voltage. (Conductances normalised by setting the conductance at -60 mV equal to 1.) Colicin K (from *E. coli*) was present in the front (*cis*) compartment at 100 ng ml⁻¹. Note that the voltage at which $g_{\text{norm}} = 1/2$, the 'switching voltage', is -31 mV, and that for voltages > -10 mV, $g_{\text{norm}} \approx 0$. The composition of the aqueous solutions are as for Fig. 1. *b*, Plot of $\ln(g_o/g_c)$ against voltage, where g_o is the fraction of channels in the open (conducting) state, and g_c is the fraction of channels in the closed (nonconducting) state. This is a replot of the data from (*a*); see ref. 25 for details. Note that the points lie on a straight line, and therefore the data are consistent with the Boltzmann relationship:

$$g_o/g_c = \exp[-nq(V - V_0)/kT]$$

From the slope of the line we see that g_o/g_c (the ratio of the number of open to closed channels) changes e -fold for every 3.7 mV. Thus n in the above equation is 6.9, suggesting that approximately 7 'equivalent gating charges' are involved in switching a channel on and off. From the intercept of the line with the abscissa, we find that V_0 , the switching voltage, is -31 mV. (In over 30 membranes, the value of n was consistently between 5 and 7; V_0 is usually around -30 mV, but occasionally values as high as -60 mV have been observed.)

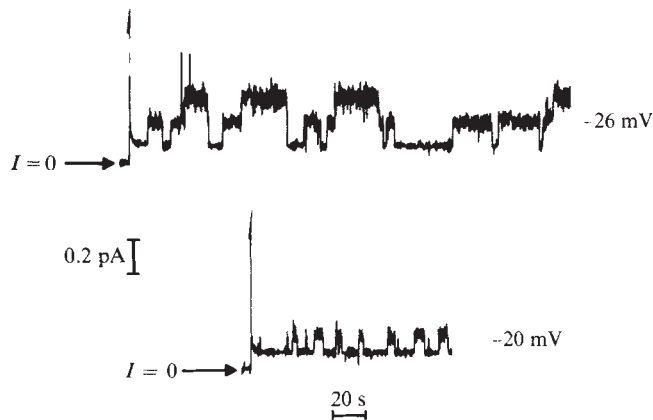


Fig. 3 Current (conductance) fluctuations due to the presence of two colicin K channels in the membrane. The large transients mark the times when the voltage was switched from 0 mV to the designated values. The conductance of a single channel in the 500 mM KCl solution is about 7.5 pS and is voltage independent. (The size of the current fluctuations is directly proportional to the applied voltage and hence is smaller at -20 mV than at -26 mV.) Note that at -20 mV ($V > V_0$) the channels tend to remain off. At -26 mV ($V \approx V_0$) there are times when both channels are on, one is on, and both are off—with a mean of about 1 channel on. For large negative voltages ($V \ll V_0$) both channels are on most of the time (data not shown). Colicin K was from *E. coli*. The membrane separates identical salt solutions consisting of 500 mM KCl + 5 mM CaCl₂ + 1 μM EDTA + 5 mM MES (all adjusted to pH 6.1 with KOH).

do not address the question of how the colicin, through interaction with its receptor in the outer bacterial membrane, reaches the plasma membrane³². Our analysis depends on its somehow doing this. We expect that colicin K and functionally related colicins (such as E1 and Ia) should be effective on suitably prepared spheroplasts and vesicles of both sensitive and resistant strains. In fact, there are already reports of this^{7,12,32-36}. The difficulties encountered in obtaining effects with these colicins on bacterial vesicles may be related to the low sensitivity to colicin action of membranes made from *E. coli* phospholipids (see above), and perhaps one function of the receptors in the outer bacterial membrane is to promote the penetration of these colicins into the plasma membrane.

Ion flux and single hit killing

An early effect on cells of E1 type colicins is the promotion of ion flux^{10,12-17}. At low colicin K multiplicity, the rate of potassium efflux from cells suspended in NaCl is directly proportional to colicin concentration; a single active molecule depletes a bacterium of 90% of its potassium in about 10 min¹⁵. Implicit in this net loss of potassium is an influx of cations (in this case Na⁺) and/or an efflux of anions (perhaps Cl⁻).

The properties of the colicin K channel observed on planar phospholipid bilayer membranes account for the *in vivo* flux data. The poor ion selectivity of the channel permits sodium and chloride, as well as potassium, movement. Furthermore, the magnitude of the channel conductance is consistent with the rates of potassium efflux observed by Wendt¹⁵, who suspended cells in 20 mM NaCl. The internal potassium concentration of the cells is about 200 mM (ref. 37). Given the selectivity of the colicin channel, it is likely that in Wendt's experiments the membrane potential of the bacteria very shortly after the insertion of colicin channels was around -58 mV. In these conditions we calculate an initial rate of potassium efflux through the colicin K channel (from *Proteus*) of approximately 10⁻¹⁸ mol s⁻¹, in agreement with Wendt's¹⁵ data, which translate into an initial rate of potassium efflux of around 5 × 10⁻¹⁹ mol s⁻¹ per killing unit. The linear dependence of conductance on colicin concentration (Fig. 4) is also consistent with Wendt's results.

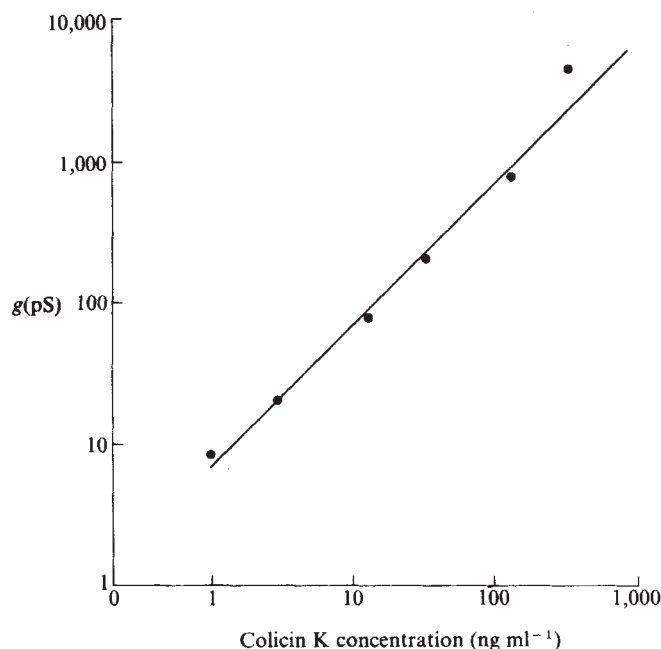


Fig. 4 Double logarithmic plot of steady-state, colicin K-induced conductance against colicin K concentration. The data were obtained on a single membrane at an applied voltage of -70 mV, with the colicin concentrations as indicated. (At this voltage the steady-state conductance has achieved a maximum value; thus the conductance plotted is the maximal conductance.) The line is drawn with a slope of 1. Conductance, and hence the number of channels, increases linearly with colicin K concentration, suggesting that each voltage-dependent channel is formed by one colicin molecule. The composition of the aqueous solutions is the same as for Fig. 1. Colicin K was from *P. mirabilis*.

A remarkable feature of colicin action is single-hit killing^{8,18-20}. For colicin K, this feature arises because one poorly ion-selective channel of a few picosiemens conductance can deplete the cell of potassium, magnesium, and other small ions within a few minutes. We do not concern ourselves here with the details and sequence of events leading to cell death following changes in the internal ionic composition (for example, inhibition of macromolecule synthesis, loss of transport systems, drop in ATP levels^{1,2}). Interestingly, however, *E. coli* survival following treatment with colicins K or E1 is greater in media containing high potassium and magnesium concentrations³⁸.

Depolarisation and disruption of active transport

A range of active transport systems are disrupted following treatment of cells with colicins of the E1 type^{3,4,39}. This phenomenon is closely related to their ability to discharge the 'energised state' of the membrane¹⁻⁷, which occurs without induction of measurable proton flux^{6,7,10}. According to the chemiosmotic theory, bacteria use the energy of oxidation to pump protons out of the cell⁵. The energy from the resulting membrane potential difference and pH gradient is used, together or separately, to drive a variety of active transport systems^{5,40}. The energised membrane state may therefore be disrupted by discharging either the membrane potential or the pH gradient.

On the one hand, it is clear that the colicin channels described here would at least partially depolarise the membrane and hence disrupt active transport; the channels are poorly selective among cations and are significantly permeable to anions. Furthermore, numerous studies on bacterial cells and vesicles are consistent with membrane depolarisation^{6,7,33,41-45}. On the other hand, given the low activity of protons in the medium ($\sim 10^{-6}$ M) and the enormous internal buffering capacity of the bacterial cell, the

colicin K channel should not significantly dissipate any preexisting pH gradient as a primary effect. The internal buffering is such⁴⁶ that it would require the entry of enough protons to bring the internal concentration to 50–100 mM (assuming no buffering) to produce a change of one pH unit. Recent measurements of the pH gradient in cells exposed to colicins K and Ia are in agreement with this expectation^{6,7,45}.

Protection by uncouplers

The normal bacterial membrane potential⁵ is of the proper sign and magnitude to open the colicin K channel. Cells are protected from colicin K action by treatments or agents (such as FCCP) which depolarise the membrane^{16,18,21}. We suggest that channel closure at low membrane potentials (Figs 2 and 3) is the basis for this protection. (This, of course, does not explain protection from colicin E2 and E3 action by these agents^{12,47}.) One possible implication of our findings is that the colicin channel might depolarise the cell so severely that the channel would close. More definitive measurements of *in vivo* membrane potentials will be required to establish the likelihood of this possibility.

Colicin K and electrophysiology

The finding that colicin K creates voltage-dependent channels in lipid bilayer membranes opens a new and potentially important avenue of research into the physico-chemical mechanisms underlying the voltage dependence of channels in excitable membranes (for example, nerve and muscle). Colicin channels offer several advantages for investigation. (1) The protein can be highly purified in large quantities. (2) Genetic selection for modified molecules is possible; indeed, we have already noted that colicins K from *P. mirabilis* and *E. coli* differ somewhat both chemically and functionally. (3) The unidirectional orientation of colicin K in the membrane, as evidenced from the voltage dependence, makes possible one-sided modification of the colicin by standard methods of protein modification. This offers a potent technique for dissecting molecular function. (4) The availability of a series of bacteriocins that may form voltage-dependent channels similar to those created by colicin K permits a comparative study of channel formers. In addition to colicins E1 and Ia, which are already identified, other likely candidates are colicin Ib, colicin A, *Staphylococcus* bacteriocin 1580, colicin S.8 and *Serratia* bacteriocin JF246 (ref. 2).

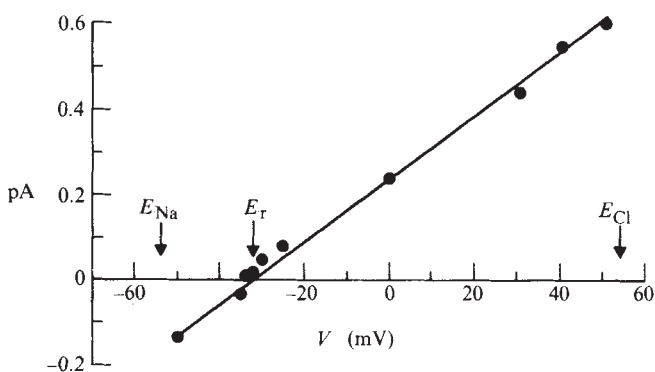


Fig. 5 Ion selectivity of the colicin K channel as determined from the reversal potential of individual channels. Across the membrane is a 10-fold gradient of NaCl concentration, which corresponds to about an 8.3-fold gradient in NaCl activity. The rear compartment contains 500 mM NaCl + 2 mM CaCl₂ + 1 μ M EDTA + 2 mM MES (pH 6.1); the front (ground) compartment contains 50 mM NaCl + 2 mM CaCl₂ + 1 μ M EDTA + 2 mM MES (pH 6.1). A very low concentration of colicin K from *E. coli* was added to the front compartment, and single-channel current jumps, such as shown in Fig. 3, were observed. The size of the current jumps is plotted as a function of membrane potential. The current jumps 'reverse' direction at $V = -33$ mV, closer to E_{Na} (≈ -54 mV), the sodium equilibrium potential, than to E_{Cl} ($\approx +54$ mV), the chloride equilibrium potential, thus showing that the channels are more permeable to Na⁺ than to Cl⁻ by a factor of about 4.

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1. Luria, S. E. in *Bacterial Membranes and Walls* (ed. Leive, L.) 293–320 (Marcel Dekker, New York, 1973).
2. Holland, I. B. *Adv. microb. Physiol.* **12**, 55–139 (1975).
3. Luria, S. E. *Annls Inst. Pasteur* **107** Suppl. 5, 67–73 (1964).
4. Fields, K. & Luria, S. E. *J. Bact.* **97**, 57–63 (1969).
5. Harold, F. M. *Curr. Topics Bioenergetics* **6**, 83–149 (1977).
6. Weiss, M. & Luria, S. E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2483–2487 (1978).
7. Tokuda, H. & Konisky, J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2579–2583 (1978).
8. Jacob, F., Siminovich, L. & Wollman, E. L. *Annls Inst. Pasteur* **83**, 295–315 (1952).
9. Fields, K. & Luria, S. E. *J. Bact.* **97**, 64–77 (1969).
10. Feingold, D. J. *Membrane Biol.* **3**, 372–386 (1970).
11. Konisky, J., Gilchrist, M. J. R., Nieva-Gomez, D. & Gennis, R. B. in *Molecular Aspects of Membrane Phenomena* (eds Kaback, H. R., Neurath, H., Radda, G. K., Schwyzler, R. & Wiley, W. R.) 193–215 (Springer, Heidelberg, 1975).
12. Nomura, M. & Maeda, A. *Zentr. Bakt.* **196**, 216–239 (1965).

13. Hirata, H., Fukui, S. & Ishikawa, S. *J. Biochem.* **65**, 843–847 (1969).
14. Dandeu, P., Billault, A. & Barbu, E. C. *r. hébd. Acad. Sci., Paris* **269**, 2044–2047 (1969).
15. Wendt, L. *J. Bact.* **104**, 1236–1241 (1970).
16. Lusk, J. E. & Nelson, D. L. *J. Bact.* **112**, 148–160 (1972).
17. Gilchrist, M. J. R. & Konisky, J. *J. biol. Chem.* **250**, 2457–2462 (1975).
18. Nomura, M. *Cold Spring Harb. Symp. quant. Biol.* **28**, 315–324 (1963).
19. Plate, C. & Luria, S. E. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2030–2034 (1972).
20. Reeves, P. *The Bacteriocins* (Springer, Berlin, 1973).
21. Jetten, A. M. & Jetten, M. E. R. *Biochim. biophys. Acta* **387**, 12–22 (1975).
22. Montal, M. *Meth. Enzym.* **32** B, 545–554 (1974).
23. Miller, C. & Racker, E. *J. Membrane Biol.* **26**, 319–333 (1976).
24. Kagawa, Y. & Racker, E. *J. biol. Chem.* **246**, 5477–5487 (1971).
25. Schein, S. J., Colombini, M. & Finkelstein, A. *J. Membrane Biol.* **30**, 99–120 (1976).
26. Läuger, P. *Science* **178**, 24–30 (1972).
27. Hladky, S. B. & Haydon, D. A. *Biochim. biophys. Acta* **274**, 294–312 (1972).
28. Stevens, C. F. *Nature* **270**, 391–396 (1977).
29. Beppu, T., Yamamoto, H. & Arima, K. *Antimicrob. Ag. Chemother.* **8**, 617–626 (1975).
30. Jesaitis, M. A. *J. exp. Med.* **131**, 1016–1038 (1970).
31. Goebel, W. F. *Proc. natn. Acad. Sci. U.S.A.* **70**, 854–858 (1973).
32. Takagaki, Y., Kunugita, K. & Matsushashi, M. *J. Bact.* **113**, 42–50 (1973).
33. Tokuda, H. & Konisky, J. *J. biol. Chem.* (in the press).
34. Bhattacharya, P., Wendt, L., Whitney, L. & Silver, S. *Science* **168**, 998–1000 (1970).
35. Kabat, J. P. & Luria, S. E. *Bact. Proc.* **62** (1970) (Abstract).
36. Obdržálek, V., Šmarda, J., Čech, O. & Adler, J. *Experientia* **25**, 331–332 (1969).
37. Epstein, W. & Schultz, S. G. *J. gen. Physiol.* **49**, 221–234 (1965).
38. Kopecky, A. L., Copeland, D. P. & Lusk, J. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4631–4634 (1975).
39. Jetten, A. M. & Vogels, G. D. *Biochim. biophys. Acta* **311**, 483–495 (1973).
40. Ramos, S. & Kaback, H. R. *Biochemistry* **16**, 848–854 (1977).
41. Gould, J. M. & Cramer, W. A. *J. biol. Chem.* **252**, 5491–5497 (1977).
42. Cramer, W. A. & Phillips, S. K. *J. Bact.* **104**, 819–825 (1970).
43. Phillips, S. K. & Cramer, W. A. *Biochemistry* **12**, 1170–1176 (1973).
44. Brewer, G. J. *Biochemistry* **13**, 5038–5045 (1974).
45. Brewer, G. J. *Biochemistry* **15**, 1387–1392 (1976).
46. Collins, S. H. & Hamilton, W. A. *J. Bact.* **126**, 1232–1244 (1976).
47. Reynolds, B. L. & Reeves, P. R. *J. Bact.* **100**, 301–309 (1969).

letters to nature

Space density evolution of quasars

ATTEMPTS to obtain interesting cosmological information from the study of quasars have been thwarted by the apparent large spread in quasar luminosities so that the conventional Hubble plot produces a scatter diagram¹. Several authors^{2–4} have used an absolute magnitude–redshift diagram to provide a standard candle and hence improved the Hubble diagram. However, little work has apparently been done on using the (M , $\log z$) plot as a cosmological indicator in its own right, in place of the Hubble diagram. It is shown here that although evolution may be zero, the uncertainty in the quasar luminosity function allows an upper limit on the quasar space density.

Figure 1 shows the (M_b , $\log z$) plot of quasars from the catalogue of Burbidge *et al.*⁵, which were chosen to have $z \leq 2$ and good photometry. The following assumptions have been made. First, quasar redshifts are of cosmological origin so that redshift is a valid indicator of distance, thus⁶

$$m = M + 5 \log D(z) + \text{const} \quad (1)$$

$$D(z) = \frac{1}{q_0} \{ q_0 z + (q_0 - 1) \{ (1 + 2q_0 z)^{1/2} - 1 \} \} \quad (2)$$

Second, the Universe can be modelled by a zero cosmological constant, zero pressure Friedmann cosmology. Third, $q_0 = 1$ has been assumed for Fig. 1, but this will be shown not to be essential.

Figure 1 shows that the luminosity of the brightest object at a given redshift is a smooth function of redshift, as has been observed by others^{2,3,7}. We propose that the upper envelope is due to a statistical sampling effect identical to the Scott effect⁸, which is, as the number of objects in a sample chosen from a given luminosity function increases, the brightest object moves

towards the bright end of the distribution, thus as z increases the volume of space sampled increases, as does the number in the enclosed sphere, so that the luminosity of the brightest object in the sphere can be expected to increase. This has been suggested recently by Sramek and Weedman⁷. This theory therefore provides a functional relationship between the luminosity of the brightest object and redshift, which should reproduce the upper envelope in the (M , $\log z$) plot, given by

$$L_{\max}(z) = f(N_z) \quad (3)$$

The model is characterised by the following assumptions: (1) the space density of quasars is constant at a given epoch, that is the cosmological principle holds. (2) Quasars are distributed according to the normalised luminosity function

$$N(M) dM = N_0 \gamma e^{\gamma M} dM \quad (4)$$

where N_0 is the number in the sample, M is the absolute magnitude and γ is a parameter to be determined observationally. This exponential function in magnitude is equivalent to a power law function in luminosity, and has been discussed elsewhere^{8–11}. (3) If N_0 objects are chosen from a distribution $e^{\gamma M}$ then the value of M for the brightest object, $M_{\max}$, is given by

$$N_0 = e^{-\gamma M_{\max}} \quad (5)$$

This result can be derived as follows. The normalised distribution in $N_0 \gamma e^{\gamma M}$. It is now postulated that the brightest object is situated where $\int_{M_{\max}}^{\infty} N_0 \gamma e^{\gamma M} dM = 1$, that is where the number of objects expected in the tail of the distribution is one, from which equation (5) follows. (4) If density evolution exists it can be characterised by

$$\rho(z) = \rho_0 e^{\alpha \tau} \quad (6)$$

where τ is the look-back time to redshift z and ρ_0 is the local

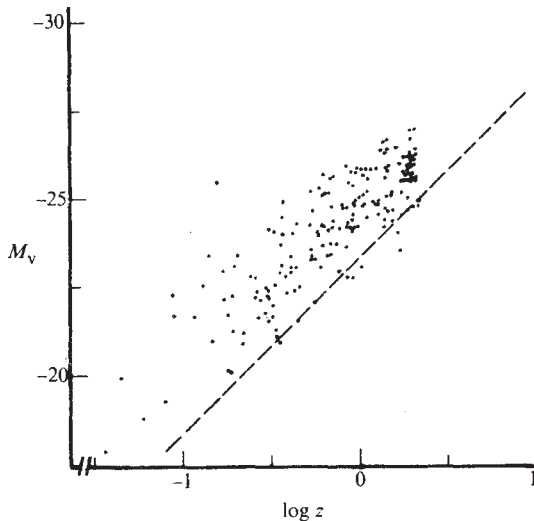


Fig. 1 Absolute magnitude of Burbidge quasars for $q_0 = 1$, as a function of $\log z$. Dotted line shows $m_v = 19$ locus.

density. This exponential law has been discussed by several authors^{9,10,13}.

The model therefore gives

$$M_{\max} - M^0 = -\frac{1}{\gamma} \ln N(z) \quad (7)$$

with

$$N(z) = \int_0^z \rho(z) dV \quad (8)$$

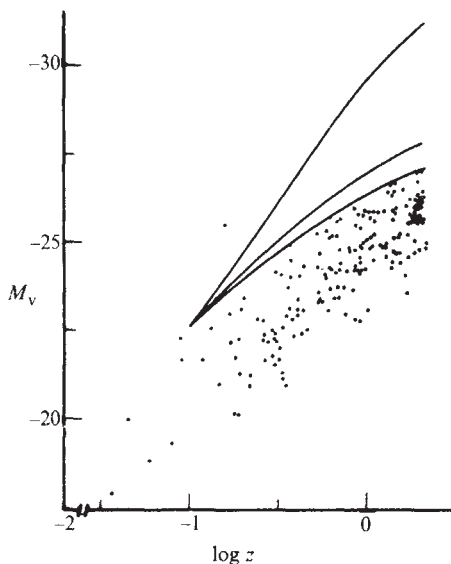
where M^0 is a luminosity cut-off to avoid divergences. The volume of space out to z is calculated from the equations given in ref. 6.

From equation (7) it follows that for low redshift, where cosmological and evolutionary effects are small

$$M_{\max} - M^0 = -\frac{1}{\gamma} \ln \rho_0 \frac{4}{3} \pi \left(\frac{zc}{H_0} \right)^3 \quad (9)$$

assuming the linear Hubble law, $R = zc/H_0$. Thus the upper

Fig. 2 Data from Fig. 1 with theoretical upper envelopes superimposed for $\alpha = 0, 2$ and 10 (see text for definition of α).



envelope in the $(M, \log z)$ plot has a gradient $-6.9/\gamma$ at low redshift. Hence the slope at low z is determined by the index of the luminosity function. Several values of luminosity function index have been given ranging from the high value^{7,9} $\gamma = 1.7$ to the low value of 1.1 (ref. 11). For the present purposes, a value of 1.4 ± 0.3 has been taken. It is to be expected that the luminosity function will be improved on in the future.

Figure 2 shows the data of Fig. 1 with three theoretical curves superimposed. The lower curve is that for zero evolution, and it can be seen that this calculation reproduces well the observational upper envelope. If a different value of the luminosity function index γ is used, it will be necessary to introduce space density evolution, as discussed by Setti and Zamorani¹¹. Thus the uncertainty in γ leads to an uncertainty in evolution. The

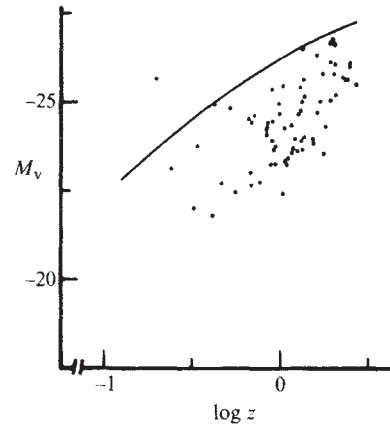


Fig. 3 Absolute magnitude of complete sample of Parkes quasars for $q_0 = 1$, as a function of $\log z$. The theoretical upper envelope for $\alpha = 0$ (no evolution) is superimposed.

result obtained for the rate of space density evolution for $q_0 = 1$ is $\alpha = 0 \pm 2$.

Table 1 compares the upper limit $e^{2\tau}$ with other evolution laws, where it can be seen that the present result gives much slower evolution. The effect of evolution on the upper envelope is shown in Fig. 2 for $\alpha = 2$ and 10, the latter being comparable to the 10^{37} law of Schmidt^{9,10}. It seems important that if the luminosity function is as flat as found by Setti and Zamorani ($\gamma = 1.1$) then evolution in a negative sense is implied, that is fewer objects in the past, but still at a slow rate. The dependence of the best-fit value of α on q_0 is:

q_0	0	0.5	1	2
α	1	0.5	0	-0.5

It can be seen from equation (9) that the zero point of the theoretical curve is determined by H_0 and ρ_0 , the local space density quasars. Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ this leads to a value of ρ_0 , which has been given in Table 2, where $\rho_0(\geq M^0)$ is the space density of objects brighter than M^0 and $\rho_0 \text{ diff.}$ is the differential luminosity function scaled to the local space density, that is the number of objects per Gpc^3 with magnitude in a unit range about M^0 . The results agree with those of Sramek and Weedman to within a factor of 2, at the bright end.

The Scott effect, which has been the basis of this letter, has had an important influence on previous determinations of quasar evolution. Apparently the Scott effect will mimic evolution by providing an excess of luminous objects at high redshift, thereby biasing the V/V_M test in exactly the same way as evolution. The effect will also show up in number-magnitude counts by simulating a 'local hole'¹². Hence it seems that what has previously been interpreted as evolution is just the result of the statistical space-sampling effect discussed above.

A further point is the behaviour of the curve beyond $z = 2.5$, where the observed number of quasars is very low (Smith¹⁴). As well as the important fact that space volume is a very low function of z , so that there is very little space volume between $z = 2.5$ and 3, it now seems that the space density is much lower than previous evolution laws have implied, which makes the expected number of objects even smaller. In addition, as Fig. 2 shows, the absolute magnitude of the brightest quasar remains nearly constant beyond $z = 2.5$, so that the objects apparently become increasingly faint with increasing redshift. This situation

Table 1 Evolutionary laws

z	τ	$(1+z)^6$	$10^{5\tau}$	$e^{2.0\tau}$
0	0	1	1	1
0.2	0.269	3	22	1.7
0.5	0.495	11.4	298	2.7
1	0.683	64	2,600	3.9
2.0	0.832	729	14,400	5.3
3.0	0.892	4096	28,840	6.0
∞	1	∞	100,000	7.4

has been discussed by Carswell and Smith¹⁵ who found that the observed number of quasars of high redshift can be accounted for assuming zero evolution. Thus it seems that the era of quasar formulation has not yet been found.

The Burbidge catalogue of QSOs has been used in the present application of this technique because it is the largest body of data available. However, due to the heterogeneity of this catalogue, selection effects might be important. It is difficult to see where such effects might arise as they would have to be redshift-dependent to affect the present analysis and because most of the objects used are radio sources, redshift dependent effects should be negligible. In addition, redshift dependence in the optical identification is unlikely, as it is the brightest objects at each redshift which are of concern and the apparent magnitude of these changes only slowly with redshift, in the redshift range used here. In any case, it was felt important to test the method on a complete and homogeneous set of data. Figure 3 shows the (M , $\log z$) diagram for Parkes objects from the Wills and Lynds¹³ catalogue, where it can be seen that the result derived from Fig. 1 is unchanged. In this work k -corrections have been neglected as they are expected to be small¹⁶. A larger sample of low redshift ($z \leq 0.1$) quasars would be very valuable to improve the determination of slope and zero-point. It has been suggested¹⁷ that steep and flat radio-spectrum quasars differ in evolutionary properties, and the present approach should be able to discriminate between the two species on those grounds, provided a good enough sample of data can be acquired.

We have found that previous determinations of quasar space density are subject to a statistical sampling effect depending on space volume. The present result is that evolution may be zero but the uncertainty in the quasar luminosity function allows an upper limit of $\rho(\tau) = \rho_0 e^{2\tau}$.

That the present method works at all must favour the cosmological nature of quasar redshifts. Also it is predicted that

absolute magnitude of the brightest object at a given redshift increases very slowly beyond $z = 3$ so that objects beyond that become increasingly faint and more difficult to detect.

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- Schmidt, M. *Astrophys. J.* **151**, 393 (1968).
- Bahcall, J. N. & Hills, R. E. *Astrophys. J.* **179**, 699 (1973).
- Kembavi, A. K. & Kulkarni, V. K. *Mon. Not. R. astr. Soc.* **181**, 19P (1977).
- Weedman, D. N. *Astrophys. J.* **208**, 30 (1976).
- Burbidge, G., Crowne, A. H. & Smith, H. E. *Astrophys. J. Suppl.* **33**, 113 (1977).
- Hawkins, M. R. S. & Martin, R. *Nature* **265**, 711 (1977).
- Sramek, R. A. & Weedman, D. N. *Astrophys. J.* **221**, 469 (1978).
- Scott, E. *Astr. J.* **62**, 248 (1957).
- Schmidt, M. *Astrophys. J.* **162**, 371 (1970).
- Schmidt, M. *Astrophys. J.* **176**, 273 (1972).
- Setti, G. & Zamorani, G. *Astr. Astrophys.* **66**, 249 (1978).
- Van Hoerner, S. *Astrophys. J.* **186**, 741 (1973).
- Wills, D. & Lynds, R. *Astrophys. J. Suppl.* **36**, 317 (1978).
- Smith, M. G. *IAU Symp.* No. 79 (1977).
- Carswell, R. F. & Smith, M. G. *Mon. Not. R. astr. Soc.* (in the press).
- Sandage, A. *Astrophys. J.* **146**, 13 (1966).
- Schmidt, M. *Astrophys. J.* **209**, L55 (1976).

Micropulsations observed by whistler-mode transmissions

DUCTED whistler-mode signals from VLF transmitters are typically Doppler shifted by ~ 0.1 Hz from the transmitter frequency. This Doppler shift has been attributed to motion of the ducts (geomagnetic field-aligned enhancements of plasma density) and measurements of the Doppler shift of NLK (18.6 kHz) signals have been used to observe relatively steady duct drifts over several hours^{1,2}, as well as duct oscillations in the Pc4–5 range³. We report here duct oscillations in the Pc3 range of a 6.6 kHz signal from a transportable VLF transmitter in Alaska, which was received at Dunedin, New Zealand⁴. Two events were observed, about six hours apart, as shown in Figs 1 and 2. For both these events, comparisons between the Doppler oscillations and ground magnetograms fit very well with current ideas about micropulsations.

Strong whistler-mode signals were received on 10 September 1973 from 13.00 to 13.30 UT showing both linear and nonlinear amplification as reported elsewhere⁵, and from 19.40 to 20.30 UT where only linear amplification occurred. Various transmission formats were sent, and the received phase at Dunedin was compared with an accurate reference phase using a 'phasogram'⁵. This is a plot, made on moving film, of phase difference against time. Thus by measuring the slope, the frequency offset, Δf , is obtained as a function of time.

Figure 1a shows this frequency offset for the interval 13.14–13.27 UT. A sinusoidal function was fitted to the data by the method of least squares, giving a period of 94.0 ± 0.4 s. At 13.00 UT one-hop delays were measured to be 1.500 and 1.455 s (ref. 5) indicating transmission in two closely spaced ducts. Dispersion analysis of whistlers occurring simultaneously with the above group delays showed the two ducts were at $L = 3.88$ and $L = 3.77$. However, during the period analysed only the signal with the 1.455 s delay was apparent, indicating that the observed oscillations occurred along the $L = 3.77$ field line.

Figure 1b shows an enlarged portion of the H trace from a magnetogram recorded at Campbell Island ($L = 4.0$), which is situated near the foot of the $L = 3.8$ field line (H is much stronger than the D component). Pulsations of 94 s period with amplitude ~ 1.3 nT are clearly present (all amplitudes are mean-to-peak).

Table 2 Local space density of quasars

M^0	$\log F$ (S.W.)	$\rho_0(M^0_3)$ (Gpc ⁻³)	ρ_0 diff	ρ_0 (S.W.)
-25.9	44.4	9.1×10^{-3}	1.4×10^{-2}	0.022
-24.9	44.0	4.1×10^{-2}	6.1×10^{-2}	0.117
-23.9	43.6	1.8×10^{-1}	2.7×10^{-1}	0.58
-22.9	43.2	8.2×10^{-1}	1.2	3.47
-21.9	42.8	3.7	5.5	20.9
-20.9	42.4	16.4	24.6	117
-19.9	42.0	73.4	110	741

Magnetic field measurements in the form of $|\partial \mathbf{B}_z / \partial t|$ were also made at Dunedin ($L = 2.7$) by recording, on the same magnetic tape as the VLF data, the voltage induced in a horizontal loop⁶ of $\sim 5 \times 10^4 \text{ m}^2$. This enabled accurate synchronisation between magnetic and VLF data. A very weak 94 s pulsation was found by spectral analysis.

As the periods of the Doppler and magnetic oscillations were the same, the oscillation was meridional and most likely fundamental with an antinode of motion at the Equator³. This is supported by calculations of the resonant frequency of oscillating field lines in a dipole field and variable plasma density⁷, where the fundamental period at $L = 3.8$ (colatitude = 30.9°) is $\sim 90 \text{ s}$.

The phase path and hence phase time, T_p , varies with latitude, tube content, and maximum equatorial displacement of the duct from its mean position, caused by the distortion of the dipole field. Andrews *et al.*⁸ showed that the electron content of the duct remains constant for Doppler fluctuations with periods $< 30 \text{ min}$. Hence we can assume that the Doppler shift is entirely due to duct movement (where magnetic field strength, and hence

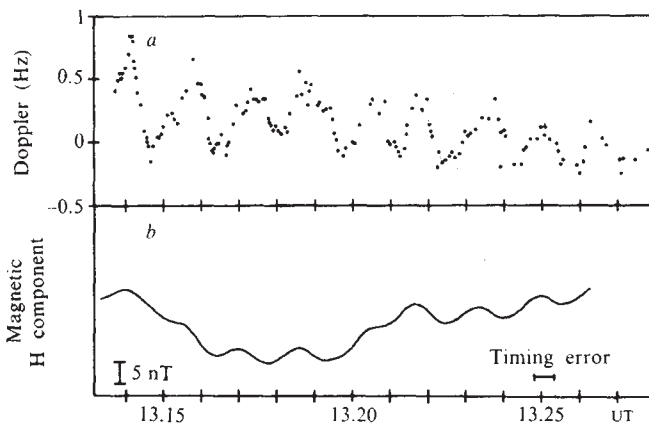


Fig. 1 *a*, Doppler shift of 6.6 kHz whistler-mode signal plotted against time for 13.14–13.27 UT, 10 September 1973. The Doppler shift is obtained by phase analysis as described in ref. 5. *b*, Magnetic **H** component scaled from a Campbell Island ($L = 4$) magnetogram. Absolute timing is $\pm 15 \text{ s}$ on the magnetogram. Magnetic variation $|\partial \mathbf{B}_z / \partial t|$ at Dunedin also shows a very weak 94 s pulsation.

refractive index and path length, change). The Doppler oscillation is assumed to be due to the radial component of motion because the azimuthal component has little effect on the phase path³.

The rate of change of phase time, dT_p/dt , equals the fractional change in frequency, $-\Delta f/f$. In its effect on the VLF wave, a small change in equatorial displacement, d (with a node near the Earth's surface) is similar to a small change in L -value of the duct in an undistorted dipole field, as the whistler-mode wave spends most of its time near the Equator. Thus by calculating the variation of phase time with latitude in a suitable model magnetosphere, we can calibrate dT_p/dt in terms of an equatorial duct velocity, v_0 , given by:

$$v_0 = R_0 \frac{dL}{dt} = -R_0 \frac{dL}{dT_p} \frac{\Delta f}{f}$$

where R_0 = Earth's radius. The peak displacement, d , is obtained by integrating v_0 over one quarter period. A model magnetosphere with a steep equatorial electron density profile proportional to L^{-5} , as the duct is near the plasmopause, gives $dT_p/dL = -0.34 \text{ s}$ and $d \sim 16 \text{ km}$. An estimate of the wave magnetic field can be obtained from this using a simplified two-dimensional model of a transverse standing Alfvén wave.

The wave magnetic field, $\mathbf{b}_x$, in the North–South plane above the ionosphere is given by:

$$\mathbf{b}_x = \mathbf{B} \frac{\partial \xi_x}{\partial z}$$

where $\mathbf{B}$ = local magnetic field, ξ_x is the meridional displacement and z is distance along a field line from the ionosphere. Assuming a dipole field and that $\mathbf{b}_x$ varies sinusoidally along the field line, with a node at the Equator, we get a displacement of 7.9 km nT^{-1} at $L = 3.8$. Hence $\mathbf{b}_x$ is just above the ionosphere ($1,000 \text{ km}$) $\sim 2 \text{ nT}$. This is slightly greater than the **H** component on the Campbell Island data.

Note that the Doppler oscillation is superimposed on a slowly varying Doppler shift. If this mean shift is also due to duct drift, then perhaps the damping of the oscillation in Fig. 1*a* can be interpreted as spatial attenuation as the duct drifts inwards from the resonant latitude. Time damping is unlikely as the Campbell Island data show no such damping in Fig. 1*b*. From the least-squares fit to Fig. 1*a*, the time for the amplitude to be halved is 408 s. The average Doppler shift over this period gives a net equatorial drift velocity of 0.45 km s^{-1} , so the half-width of the resonance region is $\sim 180 \text{ km}$. This supports models with a sharp amplitude peak at a particular latitude⁹.

The one-hop group delay for the signal in Fig. 2*a* was $1.150 \pm 0.005 \text{ s}$ which, when combined with whistler analysis gives the duct location at $L = 2.85 \pm 0.02$. Note that the frequency offset is much smaller than in Fig. 1*a*; the period of oscillation is $60.3 \pm 0.5 \text{ s}$. Magnetic variation at Dunedin Fig. 2*b* shows pulsations of this period and of about 0.2 nT amplitude very clearly. The Campbell Island magnetogram also showed barely resolved 60-s oscillations of about 0.4 nT amplitude on the **H** trace.

Calculations using a magnetospheric model with a typical equatorial density variation proportional to L^{-4} gave $dT_p/dL = -0.53 \text{ s}$ at $L = 2.85$, implying a peak displacement of $\sim 1 \text{ km}$ for the field line oscillation. This corresponds to a 0.4-nT disturbance just above the ionosphere.

Resonant field line calculations show⁷ that, for a fundamental oscillation of 60 s period, $L \approx 3.4$. Hence this observation is from below the resonant latitude and is consistent with the relatively low amplitude observed in the magnetosphere.

Cross-correlations of the digitised Dunedin magnetic variation $|\partial \mathbf{B}_z / \partial t|$ with Doppler shift for both intervals (Fig. 3*a*) show they are in phase quadrature. (Timing accuracy at Campbell Island was insufficient to allow phase comparisons with Dunedin.) The wave in the resonance region is most likely to be linearly polarised as Campbell Island **H** and **D** components seemed to be in phase. We assumed circular polarisation³ for the Dunedin magnetic data as it was far from resonance in both cases. Theoretical calculations show⁹ that the phase of $\mathbf{b}_x$ above

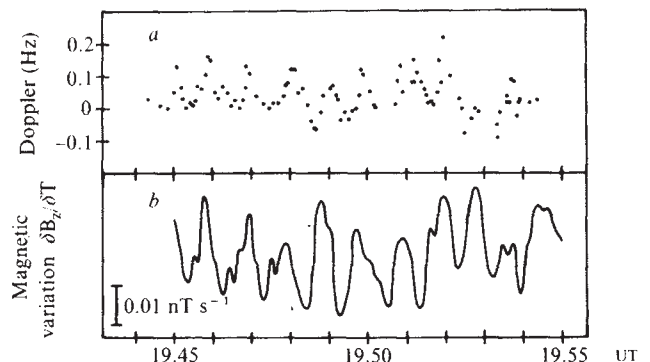


Fig. 2 *a*, Doppler shift observed in the interval 19.44–19.54 UT, 10 September 1973. Note the smaller amplitude than in Fig. 1*a*. *b*, Magnetic variation $|\partial \mathbf{B}_z / \partial t|$ recorded at Dunedin simultaneously. Pulsations of $\sim 0.2 \text{ nT}$ are very clear whereas they are barely resolvable on the Campbell Island **H** trace ($< 0.4 \text{ nT}$).

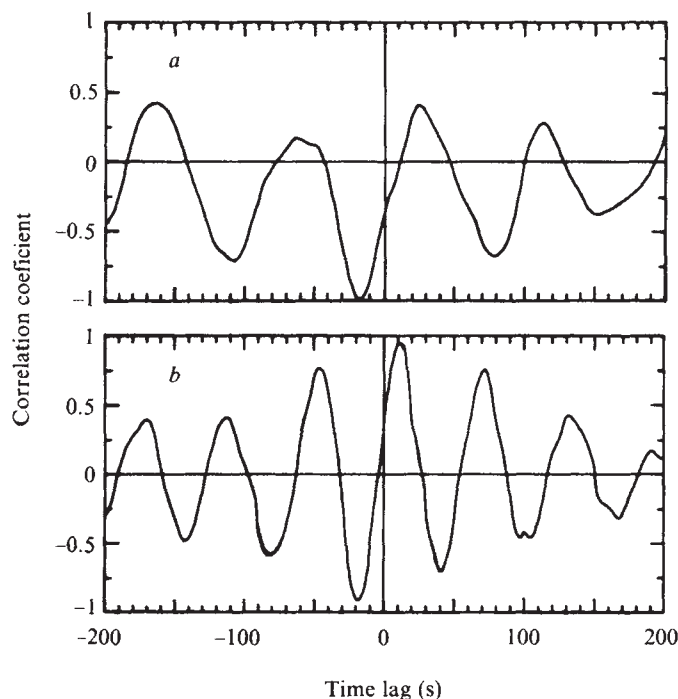


Fig. 3 Cross-correlation between Doppler shift (Δf) and magnetic variation $|\partial B_z/\partial t|$ recorded at Dunedin. Positive lag means magnetic variation leads Doppler shift. *a*, For the interval 13.14–13.27 UT a lag of 24 s is present, equal to a quarter period. *b*, The lag is 10 s for the interval 19.44–19.54 UT.

the ionosphere is relatively constant with latitude including the resonant latitude. Hence the cross-correlations are consistent with a phase rotation of 90° through the ionosphere as f and $|\partial B_z/\partial t|$ are in phase quadrature with b_z and B_z respectively. This assumes B_z has mainly the phase of the H component.

The above observations show that by phase tracking whistler-mode signals, duct oscillations in the Pc4 (and probably Pc3) range can add new information to existing ground (and satellite) micropulsation data. Indeed the fine latitudinal structure of the resonance region in the magnetosphere could not be easily obtained by other means in these latitudes. At high latitudes ($L \sim 6.3$) this structure has been studied by auroral radar¹⁰ and estimates of the width of the resonance region in terms of $\Delta L/L$ are about four times (A. D. M. Walker, in preparation) our estimate for $L \sim 3.8$.

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- McNeill, F. A. & Andrews, M. K. *J. atmos. terr. Phys.* **37**, 531–543 (1975).
- Thomson, N. R. *Planet Space Sci.* **24**, 447–454 (1976).
- Andrews, M. K. *Planet Space Sci.* **25**, 957–966 (1977).
- McPherson, D. A. *et al. Nature* **248**, 493 (1974).
- Dowden, R. L. *et al. J. geophys. Res.* **83**, 169–181 (1978).
- Offen, R. J. *Planet. Space Sci.* **20**, 1651–1659 (1972).
- Jacobs, J. A. *Geomagnetic Micropulsations* 131 (Springer, Berlin, 1970).
- Andrews, M. K. *et al. Planet. Space Sci.* (in the press).
- Hughes, W. J. & Southwood, D. J. *J. geophys. Res.* **81**, 3241–3247 (1976).
- Walker, A. D. M. *et al. Nature* **273**, 646–648 (1978).

Triplet state resonance Raman spectroscopy

AN important objective of theoretical photochemistry is to describe the interplay between the different decay channels of molecules in electronic excited states^{1,2}. Detailed information on the potential energy hypersurface is required to specify the molecular dynamics in terms of saddlepoints and activation energies along the reaction coordinate. Excited electronic states differ from the ground state because of the changes in the electronic distribution within the molecular framework. These changes are accompanied by changes in bond orders, force constants of vibration and dissociation energies. Vibrational spectra of excited states could be expected to provide important information on changes in frequencies of particular normal modes relative to those of the ground state molecule. Triplet excited states in solution have been studied by many techniques, the most common being flash photolysis in combination with optical absorption spectroscopy. Vibrational spectroscopy has to our knowledge only been reported in a single case, where one vibrational band of the lowest state of deuterated naphthalene generated by steady-state illumination was detected by infrared spectroscopy³. Recently, resonance Raman spectroscopy has been established as a powerful technique for studying shortlived species in solution⁴. This technique combines high sensitivity and time resolution with selectivity and structural information. It has been used successfully to study free radicals in solution generated by stopped flow methods⁵, electrochemically⁶, by pulse radiolysis⁴ and recently by laser flash photolysis⁷. Here we make the first report on the resonance Raman spectrum of a molecule in its triplet state generated by pulse radiolysis.

The experimental system has been described previously⁴. Excited states were produced by electron irradiation with a pulse of high energy electrons. Concentrations and kinetics of the transient species were established by optical absorption spectroscopy, and a pulsed dye laser and an optical multichannel analyser coupled to a computer were used to record the resonance Raman spectrum of the transient species.

It is well established⁸ that the lowest excited triplet state is formed on electron irradiation of solutions of organic molecules in benzene. The mechanism proposed is the formation of a radical cation–electron pair of a solvent molecule followed by fast recombination into a low-energy excited state, and the subsequent energy transfer to solute molecules exciting these into the lowest triplet state. We investigated a solution of 0.01 mol dm⁻³ of *p*-terphenyl in benzene. The maximum of the triplet–triplet absorption spectrum was found at 460 nm which agrees with the literature⁹, and the wavelength of the dye laser was tuned to approximately this value. The lifetime of the

Table 1 Vibrational wavenumbers (cm⁻¹) of *p*-terphenyl in its ground state and lowest excited triplet state

Symmetry species in D _{2h} *	Ground state†	Triplet state
A _g	1,613	1,540
	1,599	
	1,505	
	1,494	
A _g	1,284	1,350
A _g	1,195	1,227
A _g	1,038	921
A _g	1,008	
A _g	993	
	761	
	593	587

* According to ref. 10. † In CS₂, CCl₄.

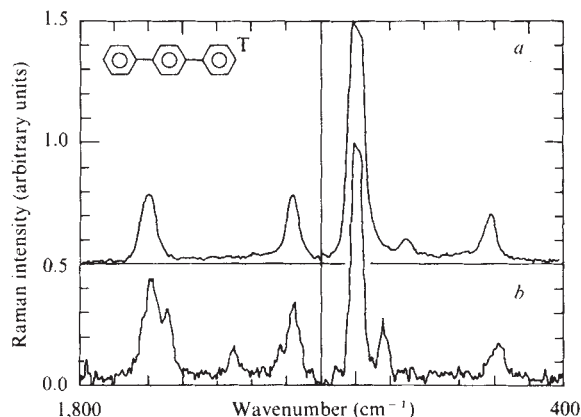


Fig. 1 *a*, Raman spectrum of a 0.01 mol dm^{-3} solution of *p*-terphenyl in benzene; *b*, resonance Raman spectrum of the same solution 600 ns after irradiation with a 30-ns pulse of 2 MeV electrons. Each spectrum recorded with two laser pulses (as indicated by the vertical line), excitation wavelength 459.0 nm.

excited species was 4.4 μs . The concentration of excited states was estimated to be $\sim 1 \times 10^{-4} \text{ mol dm}^{-3}$.

The resonance Raman spectra without and with electron irradiation are shown in Fig. 1. Table 1 lists the Raman bands found in the parent *p*-terphenyl molecule and the transient excited state. Characteristic wavenumber shifts occur in going from the parent molecule to the excited state species. Tentative assignments can be made according to the normal coordinate analysis for biphenyl by Zerbi and Sandroni¹⁰.

All bands of the transient species, with the single exception of the 587 cm^{-1} band, are assigned to totally symmetric vibrations, suggesting that A-term scattering¹¹ predominates. This seems to indicate a substantial change in geometry in going from the lowest to the higher excited triplet state. According to Zerbi and Sandroni the main contribution to the Raman band of the parent molecule at $1,284 \text{ cm}^{-1}$ originates from the ring-ring C-C stretching vibration. We believe that this band is shifted upwards by 66 cm^{-1} in the spectrum of the triplet species, which suggests an increasing bond order and a higher degree of planarity in the triplet state compared to the ground state. This result agrees with the literature: Orloff and Brinen¹² conclude from MO calculations of zero-field splitting parameters that there is a striking change in geometry in going from the ground state to the lowest triplet state of the polyphenyl-benzenes, and that a substantial shortening of the ring-ring bond occurs. Wagner¹³ suggests on the basis of spectroscopic and quenching results that in the ground state biphenyl is twisted with an interplanar angle of 25° , whereas the triplet state seems to be planar.

We have demonstrated that triplet states can be investigated by resonance Raman spectroscopy. Because of the obvious advantages of this technique compared with absorption spectroscopy, it is expected that it will become a valuable tool in the investigation of shortlived excited states, their structure and kinetics.

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- Rice, A. A. & Gelbart, W. M. *Pure appl. Chem.* **27**, 361–387 (1971).
- Henry, B. R. & Siebrand, W. in *Organic Molecular Photophysics* (ed. Birks, J. B.) Vol. 1, 153–237 (Wiley, London, 1973).

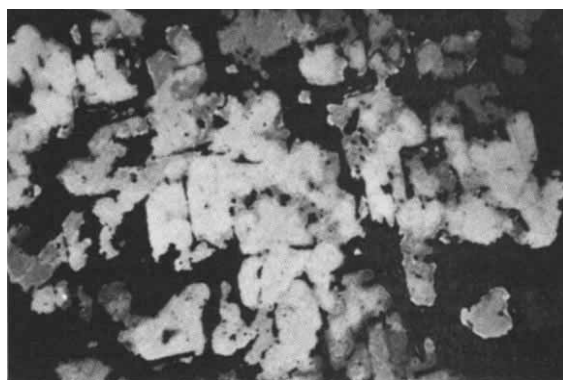
- Clarke, R. H., Kosen, P. A., Lowe, M. A., Mann, R. H. & Mushlin, R. *JCS Chem. Commun.* 528–529 (1973).
- Wilbrandt, R., Pagsberg, P., Hansen, K. B. & Weisberg, K. V. *Chem. Phys. Lett.* **36**, 76–78 (1975); Pagsberg, P., Wilbrandt, R., Hansen, K. B. & Weisberg, K. V. *Chem. Phys. Lett.* **39**, 538–541 (1976).
- Ernstbrunner, E. E., Girling, R. B., Grossman, W. E. L. & Hester, R. E. *JCS Faraday Trans. II* **74**, 501–508 (1978); *JCS Perkin Trans. II* 177–184 (1978).
- Jeanmaire, D. L. & van Duyne, R. P. *J. Am. chem. Soc.* **98**, 4034–4039 (1976).
- Atkinson, G. H., Srivastara, R. B. & Dosser, L. R. *Science* (in the press).
- Cooper, R. & Thomas, J. K. *J. chem. Phys.* **48**, 5097–5102 (1968).
- Bensasson, R. & Land, E. J. *Trans. Faraday Soc.* **67**, 1904–1915 (1971).
- Zerbi, G. & Sandroni, S. *Spectrochim. Acta* **24A**, 483–510, 511–528 (1968).
- Spiro, T. G. & Stein, P. A. *Rev. phys. Chem.* **28**, 501–521 (1977).
- Orloff, M. K. & Brinen, J. S. *J. chem. Phys.* **47**, 3999–4001 (1967).
- Wagner, P. J. *J. Am. chem. Soc.* **89**, 2820–2825 (1967).

FeNi superlattice formation by corrosion of Santa Catharina meteorite

KNUDSEN *et al.*^{1–3} have recently presented evidence from Mossbauer spectroscopy and X-ray diffraction, that an FeNi L1₀ superlattice exists in the taenite of the Cape York and Toluca meteorites. This superlattice, which had previously only been reported in neutron irradiated specimens⁴, is thought to have formed during the extremely slow cooling, 1°C Myr^{-1} , of the parent bodies of these meteorites. Here we report briefly on the discovery of the FeNi superlattice in a nickel-rich ataxite, Santa Catharina, and show that in this meteorite the ordering occurred during terrestrial corrosion.

Apart from some large idiomorphs of iron nickel phosphide (schreibersite) and a dispersion of small plates of the phosphide (rhabdite), the Santa Catharina meteorite contains two main microstructural constituents, one of which, the dark etching constituent of Fig. 1, is a face-centred cubic (f.c.c.) taenite containing 30.7 atom% Ni and having lattice parameter $a = 3.586 \text{ \AA}$. The structure of the light etching constituent has, until now, been uncertain. Thermomagnetic measurements⁵ indicated it to be a 55 atom% Ni taenite, but microprobe analysis⁶ showed that it contained up to 25 atom% oxygen. We have established that this light etching constituent consists of a dispersion of particles of FeNi spinel, Fe_2NiO_4 , $\sim 50 \text{ \AA}$ thick, in an ordered 53 atom% Ni taenite. The spinel particles comprise between one- and two-thirds (atom fraction) of this constituent. They are, however, too small to diffract X rays coherently and diffraction patterns from this constituent are single crystal patterns from the taenite. Patterns obtained using $\text{Co K}\alpha$ radiation contain reflections from all of the $\{100\}$, $\{210\}$, $\{211\}$ planes and from planes with higher indices. These reflections indicate that domains of all three orientations of the superlattice, FeNi, are present. The lattice parameter, $3.582 \text{ \AA}$, of the ordered taenite is nearly equal to that of the 30.7 atom% Ni taenite and its orientation is the same. The spinel particles can be detected in

Fig. 1 Light and dark etching constituents in the Santa Catharina meteorite. Parts of some specimens consist almost wholly of the dark constituent. Nital etch. $\times 200$.



extraction replicas, by electron microscopy and electron diffraction. They coarsen rapidly on heating and can be resolved optically after 1 h at 615 K. Reflections from the spinel particles appear in the X-ray diffraction patterns of specimens heated at 575 K for 1 h.

The terrestrial origin of the light etching constituent has been established by observing its formation in samples consisting almost wholly of the 30.7 atom%Ni taenite that were cut from the meteorite and subjected to corrosion at room temperature in water containing a small concentration of chloride ions. Figure 2 shows a section through the light etching constituent produced in a specimen corroded for 35 d. It has been detected in specimens corroded for as little as 5 d. The constituent produced by corrosion is the same in all respects as the natural material; the taenite is ordered and the compositions and electron microstructures are the same.

The mechanism by which this corrosion product is formed will be discussed in more detail elsewhere, but it is evident that the enrichment of the taenite from 30.7 to 53% Ni and the ordering of this phase, requires a degree of atomic mobility not normally available at room temperature. It is likely that this enhanced mobility is attributable to di-vacancies generated at the surface by the preferential dissolution of iron atoms⁷. Such excess vacancies would also permit the ingress of oxygen atoms to form the spinel.

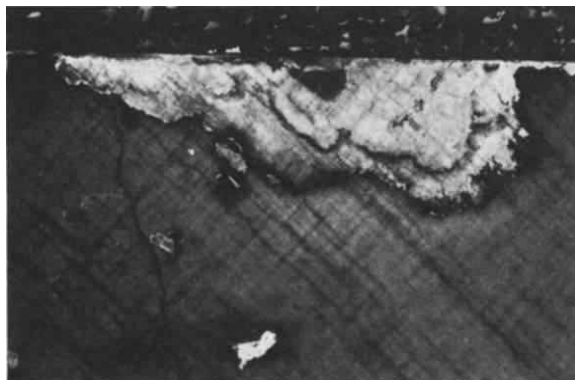


Fig. 2 Light etching constituent produced by corrosion. Nital etch. $\times 200$.

The formation of the $L1_0$ superlattice during corrosion obviously means that care must be taken when using the presence of this superlattice as an indication of the thermal history of meteorites. However, it is not expected that the taenite lamellae in octahedrites would be susceptible to this type of corrosion process while the low nickel kamacite is present. Even when the kamacite has corroded away the high nickel concentrations, which are produced in the outer layers of the taenite during the precipitation of the kamacite, would corrode much more slowly than the taenite of Santa Catharina.

It is curious that the lattice parameter of the nickel-rich ordered taenite in Santa Catharina is the same as that measured by Knudsen *et al.* in Cape York and Toluca. In these meteorites, the taenite lamellae were found to contain a disordered f.c.c. phase as well as the superlattice, the lattice parameters of both phases being $3.582 \pm 0.002 \text{ \AA}$. Comparison with published parameters⁸ showed that the disordered phase contains approximately 28 atom%Ni and it was proposed that these two phases occur in varying proportions in the nickel concentration gradient in the taenite. The concentration gradient is considered to be 'frozen in', but within each volume element short-range diffusion of nickel has permitted the development of the two-phase structure. In this model the two-phase region is identified with the dark etching zone of so-called cloudy taenite observed in many octahedrites, whereas the clear taenite rim is considered to consist almost wholly of superlattice.

Knudsen *et al.* consider that the domains of ordered taenite in the two-phase region have the composition FeNi, but published lattice parameters of iron nickel alloys indicate that ordered taenite with $a = 3.582 \pm 0.002 \text{ \AA}$ contains $53 \pm 2 \text{ atom\%Ni}$. Our microprobe analyses of Santa Catharina confirm that ordered taenite with this lattice parameter contains more than 50 atom%Ni. These analyses indicate that the light etching constituent contains varying proportions of Fe_2NiO_4 and taenite containing $\sim 55 \text{ atom\%Ni}$.

It is surprising that a single lattice parameter was observed in the Ni concentration gradients of Cape York and Toluca. The nickel concentration in the taenite at the taenite-kamacite interface is determined by the composition of disordered taenite in equilibrium with kamacite at the temperature at which growth of the kamacite ceased. The concentration of nickel in ordered taenite in metastable equilibrium with disordered 28 atom%Ni taenite is expected to be below this upper limit. Thus, the concentration gradient should contain a two-phase region in which the lattice parameter of the ordered taenite is constant, and a single-phase region, in which the lattice parameter varies between this value and that of taenite in equilibrium with kamacite. The range of nickel concentrations in this single-phase region indicated by the error limits of the parameters measured by Knudsen *et al.* is $53 \pm 2 \text{ atom\%Ni}$, implying that the lower limit of the clear taenite is at 51 atom%Ni. This conclusion conflicts with other measurements^{9,10} which indicate a lower limit of 40 atom%Ni.

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Received 9 August; accepted 28 September 1978.

1. Petersen, J. F., Aydin, M. & Knudsen, J. M. *Phys. Lett.* **62A**, 192-194 (1977).
2. Albertsen, J. F., Aydin, M. & Knudsen, J. M. *Physica Scripta* **17**, 467-472 (1978).
3. Albertsen, J. F., Jensen, G. B. & Knudsen, J. M. *Nature* **273**, 453-454 (1978).
4. Pauleve, J., Dautreppe, D., Langier, J. & Neel, L. *C.r. hebdom. Séanc. Acad. Sci., Paris* **254**, 965-968 (1962).
5. Lovering, J. F. & Parry, L. G. *Geochim. cosmochim. Acta* **26**, 361-382 (1962).
6. Lovering, J. F. & Anderson, C. A. *Science* **147**, 734-736 (1965).
7. Pickering, H. W. & Wagner, C. J. *Electrochem. Soc.* **114**, 698-706 (1967).
8. Ramsden, A. R. & Cameron E. N. *Am. Mineral.* **51**, 37-55 (1966); **51**, 1544-1545 (1966).
9. Scott E. R. D. *Geochim. cosmochim. Acta* **37**, 2283-2294 (1973).
10. Lin, L. S., Goldstein, J. I. & Williams, D. B. *Geochim. cosmochim. Acta* **41**, 1861-1874 (1977).

Earthquake precursors from Earth tilt observations corrected for rainfall

AN Earth tilt meter at Wellington, New Zealand (lat $41^\circ 14' \text{S}$, long $174^\circ 47' \text{E}$) has been operating since 1972 in old underground tunnels which are about 15 m below the surface. Installation transients, at first of the order of several microadians per year, have decayed slowly and are now about $0.3 \text{ } \mu\text{rad yr}^{-1}$. As is well known^{1,2} rainfall and evaporation, in all but very deep sites, usually completely obscure any obvious relationship with tectonic events. Wood and King³ have made one unsuccessful attempt to deal with this problem but the largest earthquake occurring was only of magnitude 5.2. A new approach, described here, has yielded significant correlations of tilt with two, and possibly three, earthquakes of magnitudes 5 to 6.2. The precursor times are in fair agreement with Rikitake's⁴ equation.

Our approach has been to deal solely with monthly mean values of tilt, rainfall and the other meteorological elements involved. After correcting for both the exponential installation transient and average seasonal variation the monthly tilt departure from normal, T_0 , correlates significantly with the monthly

rainfall minus evaporation departures, $(R - E)$, for the previous 5 months. This leads to a tilt correction of the form:

$$T\alpha = a(R - E)_0 + b(R - E)_1 + c(R - E)_2 + d(R - E)_3 \\ + e(R - E)_4 + f(R - E)_5 + gt_0 + ht_1 + jP_0$$

where the suffix refers to the n th month before the one in question, t is the monthly mean temperature departure and P that for atmospheric pressure. Meteorological data were supplied by a nearby weather station.

The coefficients a – h were optimised to produce the highest correlation coefficient between the values T_α and T_0 . Other possibilities were also tried, like extending the series for more than 5 months into the past, but the highest correlation coefficients obtainable were $r = 0.89$ and 0.83 for the N–S and E–W tilt components respectively. This was done for the entire period of the study, 1974–78, and indicates, for instance, that only $100(1 - r^2) = 21\%$ of the N–S variations observed are due to non-meteorological causes, presumably tectonic.

The residual tilt, $T = T_0 - T_\alpha$, is shown in Fig. 1, together with earthquakes of $M \geq 5$ occurring near enough to the station to be of significance. Figure 2 shows the epicentres, magnitudes, and dates of all normal earthquakes $M \geq 5$ occurring within about 150 km during the period analysed. The NZ Seismological Observatory supplied the seismological data⁵.

The fit of the tilt anomalies with the two largest nearest earthquakes labelled B and C is remarkable; in each case the

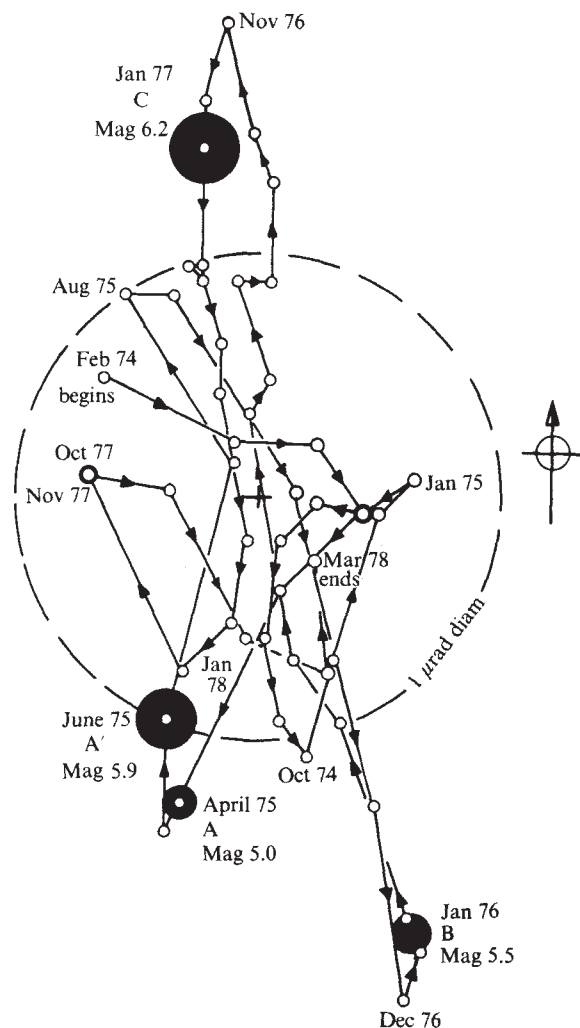


Fig. 1 Wellington Residual Earth Tilt 1974–78. Small open circles are monthly tilt values with only significant months labelled. Large solid circles are significant earthquakes lettered to correspond with Fig. 2.

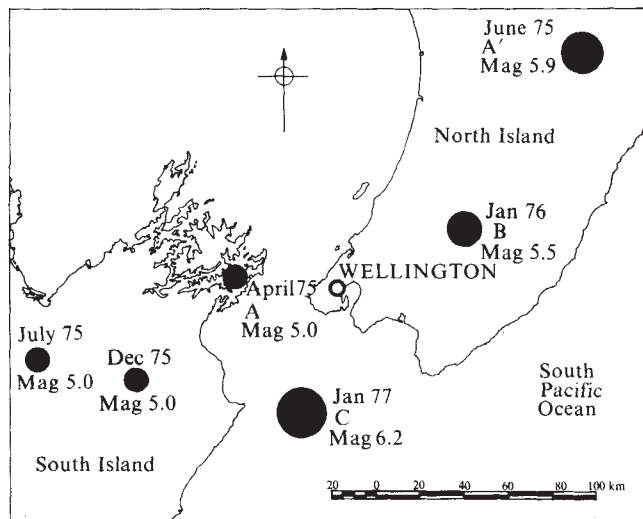


Fig. 2 Epicentres of all normal earthquakes of magnitude $M \geq 5$ within about 150 km of the station, during the period covered by Fig. 1. The letters refer to the corresponding earthquakes in Fig. 1.

earthquake occurred after the tilt anomaly began to return to normal and the precursor time, while not agreeing exactly with Rikitake's⁴ equation, is, nevertheless, longer for the larger earthquake even though this has the smaller anomaly. A change in the direction of tilt a month or so before the earthquake has been mentioned by Scholz *et al.*⁶ in connection with Japanese earthquakes. As well as showing this effect it is also to be noted that the anomaly directions tend to be away from the future epicentres.

However, there are three possible interpretations for the first and smallest anomaly peaking in May 1975. First, it might be of no significance since it is only slightly greater than the general random walk background or it could be correlated with either earthquake A or A'. Earthquake A' was of magnitude 5.9 and, coming in June 1975, would provide a better fit because it was then past the tilt maximum; the tilt direction is also a better fit than for earthquake A, which was smaller and nearer. Earthquake A' was probably too far away to be detected, however. None of the other normal earthquakes were near enough or large enough to be regarded as significant. One deep earthquake, not shown, had a depth of 128 km, a magnitude of 5.9, and occurred in January 1975 having its epicentre near B. No tilt anomaly at this time is apparent and this is consistent with theories of dilatancy which suggest that a deep earthquake may not produce precursors.

Restricting the situation to normal earthquakes of $M \geq 5.5$ within 100 km a statistical analysis based on a Poisson distribution has yielded a probability of 3.1×10^{-3} that the overall agreement is due to chance. Thus the apparent precursory tilts are probably genuine, at least in the two clearest cases. A forthcoming damaging earthquake ($M > 6.5$, say) at a favourably situated epicentre should produce a definite detectable precursory tilt.

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- Gerard, V. B. *Phil. Trans. R. Soc. A* **274**, 311–321 (1973).
- Allen, R. V., Wood, D. M. & Mortensen, C. E. *Phil. Trans. R. Soc. A* **274**, 219–222 (1973).
- Wood, M. D. & King, N. E. *Science* **197**, 154–156 (1977).
- Rikitake, T. *Earthquake Prediction* 295 (Elsevier, Amsterdam, 1976).
- New Zealand Seismological Reports, E Series, NZDSIR, Geophysics Division.
- Scholz, C. H., Sykes, L. R. & Aggarwal, Y. P. *Science* **181**, 803–809 (1973).

Sea level–air temperature correlations near a coastal zone

A NUMBER of naturally occurring chronometric records, such as the growth rings of trees¹, distribution of pelagic fish scales preserved in anaerobic sediments² and the thickness of annual laminations of these sediments³, have been correlated with time series of climatological variables. I have previously introduced sea level as one predictive variable of diatom abundance⁴; here I propose sea level as another predictive variable which can be used to estimate air temperature near the coast of San Diego (32°43'N 117°10'W) and Los Angeles (33°56'N 118°24'W). Although historical data series of sea level and air temperature have been correlated with several atmospheric and oceanic variables in the past⁵, no correlation between them has, as far as I know been reported previously.

The causes of long-term (months, years) variations in sea level have been enumerated by Montgomery⁶, and Lizitzin⁷. These are: (1) meteorological (atmospheric pressure, wind, precipitation and evaporation); (2) oceanographic (water density and currents); (3) river discharge; (4) the eustatic contribution; and (5) the nodal tide with the period of 18.61 yr. Factors (3) and (5) can be eliminated for the San Diego region; river discharge is negligible, and the effect of the nodal tide is expected to be very small as one of the nodal lines is located at 35°N (ref. 8), which is very close to the location under study.

Stewart *et al.*⁹ investigated data recorded at several stations along the coast of North America and reported that the anomalously warm sea surface temperatures during the 1957–58 period coincided with anomalously high sea levels. Roden¹⁰ verified this finding and reported that there was a significant correlation between monthly anomalies (deviation from long-term mean) of sea level and monthly anomalies of sea surface temperatures. Changes in sea level, during two decades, 1948–57 and 1958–69, have been studied by Namias and Huang¹¹. They reported that the largest cause of variation was thermal, accounting for 3.7 cm, followed by the overall dynamic effect, 1.0 cm, and atmospheric pressure differences (the inverted barometer effect) 0.6 cm.

It is also well known that at several coastal stations of North America, including San Diego, sea surface and air temperatures are significantly correlated¹². These findings are sufficient to hold as *a priori* that changes in sea level should be reflected in air temperatures.

Data plotted in Fig. 1 show that this is the case for both locations. Statistical methods used in obtaining Table 1 can be found in ref. 13. Briefly, average skill index calculated from N number of data points, $S_H(N)$, can be written as the sum of two

Table 1 Trends and correlation coefficient (r), skill index, S_H (per cent of explained variance, r^2) and artificial skill, S_A

Location	Period	AT trend	SL trend	r	S_H	S_A
San Diego	1906–76	0.03 °C yr ⁻¹	0.19 cm yr ⁻¹	0.67	0.42	~0.04
Los Angeles	1936–75	0.04 °C yr ⁻¹	-0.03 cm yr ⁻¹	0.74	0.54	~0.03

Values calculated from anomalies of air temperature and sea level after both variables had been de-trended.

For statistical methods see text and ref. 13. For trends and variability of yearly mean sea level calculated for various locations, see ref. 14.

terms: $S_H(N) = S_T + S_A$ where S_T is the true skill index, and S_A the artificial skill which is due to any error in sample correlations which will lead to an increase of skill above the true skill index, S_T , and depends on the number of data points: $S_A = K/N$ where K depends on time scales. The method of calculating S_H (1.0 = perfect correlation, 0.0 = no correlation) is the same as for calculating the per cent of explained variance, r^2 , where r is the correlation coefficient. (For various ways of calculating S_A , see ref. 13). As can be seen in Table 1, skill indices for both locations are significantly greater than the artificial skill indices; that is, they are significantly correlated. Annual anomalies of both variables have been calculated after the effect of their respective trends have been removed.

It has been customary to adjust sea level data to the effect of the atmospheric pressure (theoretically, at least, sea surface should be depressed by 1 cm for every rise in atmospheric pressure of 1 mbar), however, this is questionable "since variation in atmospheric pressure is associated with change in the direction and velocity of the wind, which is one of the most important factors contributing to the fluctuations in sea level"⁷. The skill index, S_H , calculated between sea level where the 'effect' of the atmospheric pressure has been removed and air temperature at San Diego is 0.37 for the 1929–73 period, which is still higher than the artificial skill of 0.04, and is thus also predictable.

An important question is whether the use of seasonal rather than annual anomalies could improve the correlations presented in Table 1. For example, in several locations in Scandinavia cold winters are often followed by warm summers¹⁵; so that the annual anomalies of air temperatures might not be representative of the major changes at these locations. However, according to Namias¹⁶, this is not true for the southern California coast. That is, negative or positive seasonal anomalies of air temperatures often persist throughout the year. The average skill index, S_H , between the seasonal anomalies of air temperature and sea level for the San Diego region during 1906–76 period is 0.29 (artificial skill, S_A , is <0.01) which is much smaller than the skill index calculated from the annual anomalies (0.42, Table 1)

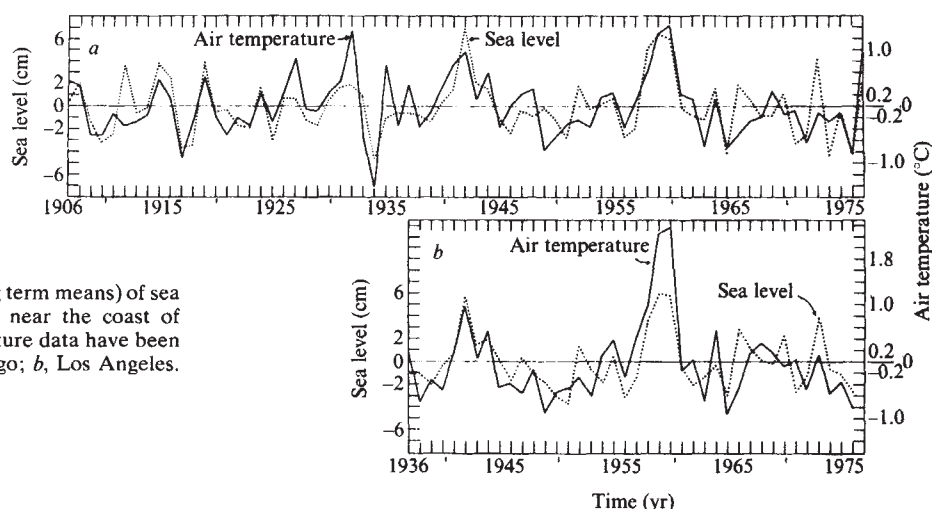


Fig. 1 Yearly anomalies (deviation from long term means) of sea level and air temperatures at two locations near the coast of southern California. Only limited air temperature data have been recorded at Los Angeles Airport. *a*, San Diego; *b*, Los Angeles.

indicating that the use of seasonal anomalies would not improve the correlation.

At best there can only be an indirect cause-effect relationship between air temperature and sea level. However, the remarkable correlations shown in Table 1 strongly suggest that the dominant mechanisms causing fluctuations are the same. Two possible mechanisms for these fluctuations are changes in solar irradiance and wind. Previous research indicates that the most likely mechanism in this case is changes in wind systems.

Reid *et al.*¹⁷ have reported a significant correlation between the monthly anomalies of the northerly wind component and of sea surface temperatures during spring and early summer months. Namias and Huang¹¹ associated the higher sea surface temperatures and higher sea levels found during 1948–57 as compared to 1958–59 period with a lesser north-to-south wind stress. After an extensive study of current, wind and sea level variations off the Oregon coast Smith¹⁸ reported a high correlation between southwards winds and sea level. Climatological properties of the air characteristic of a location are transported when the wind deviates from its normal flow¹⁹, thus, increased northerly winds are expected to result in lower air temperatures in this region.

This hypothesis may be an oversimplification of the complex meteorological processes which make up climate. However, from a pragmatic point of view, the existence of such a significant correlation between these two variables is of great value to the investigators of various disciplines.

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1. Fritts, H. C. *Mon. Weath. Rev.* **93**, 421 (1965).
2. Soutar, A. & Isaacs, J. D. *Calif. Mar. Res. Commun. CalCOFI*, **13**, 63 (1969).
3. Soutar, A. & Crill, P. A. *Geol. Soc. Am. Bull.* **71**, 631 (1977).
4. Tont, S. A. *Science* **194**, 942 (1976).
5. Saur, J. F. T. *Fish Bull.* **70**, 619 (1972).
6. Montgomery, R. B. *J. mar. Res.* **1**, 165 (1936–1938).
7. Lizitzin, E. *Sea-Level Changes*, 166 (Elsevier, New York, 1974).
8. Lizitzin, E. *Sea-Level Changes*, 39–41 (Elsevier, New York, 1974).
9. Stewart, H. B., Zetler, B. D. & Taylor, C. B. *Tech. Bull.* 1–11 (U.S. Coast and Geodetic Survey, Washington, D.C. 1958).
10. Roden, G. I. *J. geophys. Res.* **65**, 2809 (1960).
11. Namias, J. & Huang, J. C. K. *Science* **177**, 351 (1972).
12. Roden, G. I. *J. appl. Meteorol.* **5**, 3 (1966).
13. Davies, R. E. *Geophys. Astrophys. Fluid Dynamics* **8**, 245 (1977).
14. Hicks, S. D. & Crosby, J. E. *NOAA Tech. Memo. NOS 13*, 5 (National Ocean Survey, National Oceanic and Atmospheric Administration, Rockville, Maryland).
15. Namias, J. *J. Meteor.* **14**, 235 (1957).
16. Namias, J. *Mon. Weath. Rev.* (in the press).
17. Reid, J. L., Roden, G. I. & Wyllie, J. G. *Calif. Coop. Oceanic Fish. Invest. Prog. Rep.* **51** (1958).
18. Smith, R. L. *J. geophys. Res.* **79**, 435 (1974).
19. Namias, J. *Meteorol. Monogr.* **2**, 20 (1953).



Fig. 1 a, Attached dextral specimen of *Janua* sp. on brown seaweed. Specimens are of early Holocene age. (Gyre Core 343, 985–1,000 cm, Orca Basin, northern Gulf of Mexico) $\times 26$. b, Higher magnification view of single *Janua* sp. showing details of surface ornamentation of transverse lines and spiral ridges $\times 63$.

of these cores, which consists of black, organic-rich, fetid mud, resulting from the anoxic bottom conditions within this basin. The seaweed fragments are small (<4 mm) and the scanning electron microscope shows them to have clear cell walls. The core, located at $26^{\circ}54' N$, $91^{\circ}21.5' W$, was raised from a water depth of between 2,400 and 2,450 m and is 12 m in length. The exact depth has not yet been established because sound velocity of the brine in the basin has yet to be determined. As expected, the microfossil sequence preserved in this core is quite atypical for the Gulf of Mexico, consisting of persistent, relatively abundant, and well preserved planktonic foraminifera and radiolaria. Late Quaternary microfossil sequences outside this anoxic basin in the Gulf of Mexico differ from that of the Orca Basin by exhibiting planktonic foraminiferal assemblages that have undergone varying degrees of dissolution; pteropod assemblages occurring only spasmodically and exhibiting conspicuous dissolution and an almost complete absence of radiolarian assemblages². Thus, the unusual sedimentary geochemical conditions within the Orca Basin have created an opportunity to study more complete microfossil sequences than in other areas of the Gulf of Mexico. The sequence contains persistent *Globorotalia menardii* and *Globorotalia tumida* indicating a Holocene age ($t < 10,000$ yr BP)².

We do not know of any previous documentation of seaweed in deep-sea sediments, let alone that with attached fossil organisms. Seaweed is normally very rapidly recycled within the food chain. The attached organisms (Fig. 1a and b) are calcareous, dextrally coiled polychaetes of the family Spirobridae. The specimens have been examined (by Richard Beckwith) and identified as *Janua* sp. based on shell and operculum characters. The specimens (Fig. 1b) show conspicuous transverse lines and prominent spiral ridges. The seaweed is a brown form and is probably *Sargassum* which is commonly found floating in warm waters in the Atlantic³. Seaweed such as this is apparently commonly deposited in the Orca Basin, as Jeffrey and Schink (personal communication) have discovered a large amount of brown seaweed in a box core from the Orca Basin. It is apparent therefore that the seaweed sank rapidly to the floor of the Orca Basin without being consumed by mid-water organisms, and was preserved in the Holocene sediments because of the anoxic conditions and a lack of benthonic organisms within the basin. Our microfossil examination of the samples revealed an almost

Fossil Holocene seaweed and attached calcareous polychaetes in an anoxic basin, Gulf of Mexico

DURING examination of the planktonic foraminiferal assemblages in a piston-core of black, anoxic sediments (Gyre, 77-G-13, Leg 1, 5-K) of probable early Holocene age from the Orca Basin, northern Gulf of Mexico, we discovered preserved, brown seaweed and calcareous polychaetes from a core depth of 985–1,000 cm. We know of no previous documented case of preserved seaweed in deep-sea sediments, especially with attached organisms, and we describe this material here.

The Orca Basin is a 400 km² depression in the continental slope of the northern Gulf of Mexico, which contains anoxic, hypersaline (about 250 g kg⁻¹) water in the bottom 200 m (ref. 1). The salt is considered to be derived by leakage from nearby diapiric structures¹. Two piston-cores have been taken using the RV Gyre, and we have examined microfossil samples from one

complete absence of benthonic foraminifera and other benthonic microfossils, in contrast to very rich planktonic microfossil sequences (planktonic foraminifera, pteropods and radiolaria) throughout the core. We observed only about four specimens of benthonic foraminifera in the core samples examined. It is unclear whether these specimens somehow managed to live in the Orca Basin or whether they were transported in. The microfossil sequences in the core exhibit no evidence of bottom-sediment transportation. The benthonic foraminifera, however, are so rare they could have been transported in by unusual mechanisms, including seaweed transportation.

We thank Robert Tompkins and Dr David Schink for providing samples from the core. The core was collected by Robert Tompkins. We also thank Richard Beckwitt for his identification of the polychaetes. Donald Scales operated the scanning electron microscope. This research was funded by NSF grant no. OCE75-21262.

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1. Shokes, R. F. *et al. Science* **196**, 1443–1446 (1977).
2. Kennett, J. P. & Huddleston, P. *Quat. Res.* **2**, 38–69 (1972).
3. Fuller, H. L. *College Botany* (Holt, New York, 1954).

Detection of bubbles in decompression sickness

AMATEUR diving and industrial demands have changed decompression sickness from being a specialised problem to becoming a health risk for many thousands of people. Rising medical standards and the recognition of bone damage¹ have resulted in increasingly stringent diving regulations, but the devising of suitable decompression schedules is still largely empirical; our best strategy is still to avoid sickness by accepting grave restrictions on diving procedures (such as decompression for a week or more²). It is important to find the earliest events involved, particularly as it is now generally accepted that the condition arises from supersaturated gas separating in the tissues; separation precedes overt symptoms and may remain symptomless³ and experimentally at least, gas does not readily separate without some nucleus^{4,5}. Methods are required applicable to man which can detect separated gas as such, and not by the physiological effect it produces, as early as possible. Various acoustic methods have been used. Bubbles moving in blood vessels have been successfully detected by the Doppler method^{6,7} in experiments confirming their significance and presymptomatic existence³. However, this method cannot recognise stationary bubbles. We describe here a method using ultrasonic imaging that will make it possible to detect moving or static bubbles down to the required limits, in a chosen plane in the body, and hence to study at the earliest stages the location, development and movement of bubbles in a variety of tissues after subjection to decompression procedures.

The first such method used was an acoustic-optical method⁸ which proved lacking in resolution. An alternative, first explored by MacKay⁹, uses pulse-echo ultrasonic techniques. The method can, in principle, recognise gas at any site, static or in motion. It should therefore be possible to identify the sites of origin, and analyse the processes of bubble formation. Given this knowledge, one could hope to avoid decompression sickness without the need for tedious (and sometimes damaging) trials.

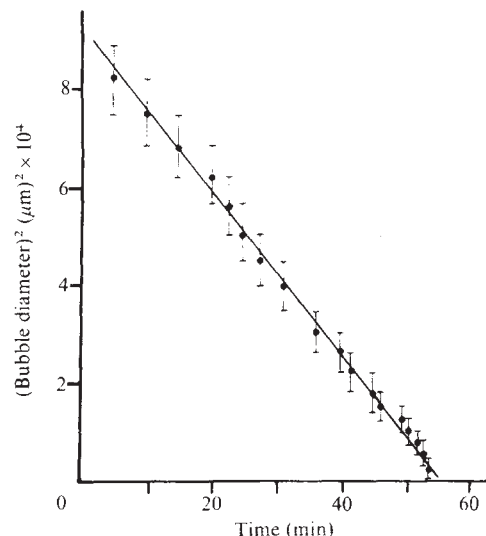
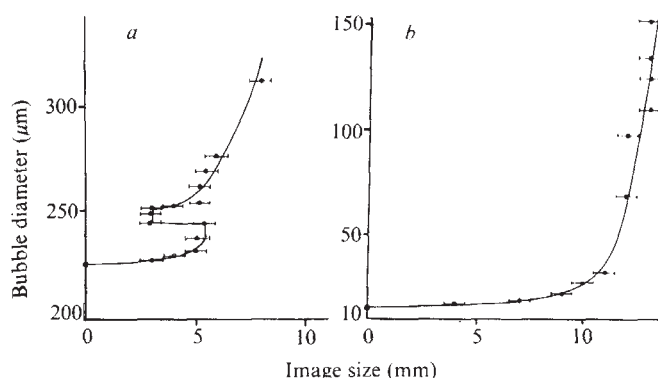


Fig. 1 Plot of bubble decay in degassed water, verifying the Epstein-Plesset equation which relates the square of bubble radius to time. A least-squares linear regression analysis gave a correlation coefficient of 0.998. The error bars represent $\pm 10 \mu\text{m}$ in measuring bubble diameter.

Using a pulse-echo system operating at 7.5 MHz, Rubissow and MacKay¹⁰ reported routine observation of bubbles of 2–5 μm diameter and even down to 0.5 μm in optimum conditions. Furthermore, they suggested that the actual diameter of such bubbles could be measured. The claim that such small bubbles could be detected has been challenged on theoretical grounds but without repeating the experiments^{11–13}. At 7.5 MHz the wavelength of sound in tissue is 200 μm and from the relationship between target radius and back-scattered sound intensity¹⁴ this detection limit seemed unlikely. In view of this controversy and the promise of this technique for the study of decompression sickness, an 8-MHz pulse-echo system has been developed: at this frequency, bubbles can be detected down to 10 μm diameter, measured microscopically, but not below; bubble size cannot be obtained from image size for bubbles $< 300 \mu\text{m}$ in diameter; and 10 μm diameter bubbles seem likely to be the smallest stable *in vivo*.

The pulse-echo system was based on two commercially available units, which after modification, provided the basic amplitude and brightness modulated displays, with sound frequency 8 MHz and pulse repetition frequency 2 kHz; using a 5-mm

Fig. 2 Ultrasonic image size as a function of gas bubble diameter, plotted by simultaneously observing the decay of the gas bubble ultrasonically and by optical microscopy. *a*, Image size measured using a low (45dB) gain. *b*, Image size measured using a high (80dB) gain. The frequency of sound used was 8 MHz corresponding to a wavelength of 187 μm (the acoustic coupling medium being water).



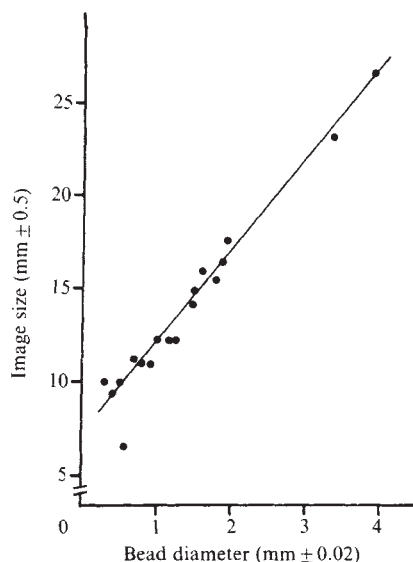


Fig. 3 Ultrasonic image size as a function of target size, measured using spherical glass targets, size range 0.4–3.5 mm diameter. The glass spheres were held in the centre of the ultrasound beam by a fine glass thread. The bead diameters were measured using a microscope fitted with a calibrated graticule. The ultrasonic image sizes were all measured from the photographic record of the images recorded from the glass beads.

diameter probe. The probe is focused with a plano-concave epoxy lens, fixed directly to the face of the PZT-5A transducer. A simple sector scan mode is used, at a frequency of 1 Hz, with the target coupled acoustically to the transducer with water. The ultrasonic images are displayed on a variable persistence oscilloscope. (Full details of the system will be published elsewhere.)

To determine the detection threshold of this system the decay of small gas bubbles in water has been observed simultaneously by the ultrasound system and by optical microscopy, which also allowed the relationship between bubble radius and image size to be determined. The test bubbles were confined in a small depression in a bubble-free block of 4% gelatin, gelatin being both acoustically and optically transparent. The block, held by a hypodermic needle, was positioned in the ultrasound beam with a micromanipulator, and coupled acoustically to the probe by degassed, distilled water. Single bubbles, of ~300 µm diameter, were formed in the cavity using a microelectrode and syringe. Microscopically such bubbles decayed over a period of several hours, and in over 24 h in air-saturated water. Their diameter could be measured to an accuracy of ±5 µm. Bubble decay followed the Epstein-Plesset equation¹⁵, which, ignoring surface tension effects, has the approximate solution:

$$(R/R_0)^2 = 1 - (2x/R_0^2)t,$$

where R = bubble radius; t = time; and x = constant. Although the equation is expected to be inaccurate for small bubbles no appreciable deviation was found in this study (Fig. 1).

The ultrasound images of the bubbles were recorded automatically every 2 s at selected periods during their decay, and continuously when their diameter fell below 50 µm. Under the microscope, when the diameter reached 10 µm the bubbles apparently seemed to vanish suddenly, even in the presence of surfactant, detergent or when the surrounding medium was air saturated. A plot of bubble diameter against image size (Fig. 2) shows that, in optimum conditions (Fig. 2b), the detection limit of the ultrasound system is close to 10 µm, but there is no simple relationship (Fig. 2a) for bubbles with a diameter <300 µm. This result was expected from consideration of the scattering cross-sections^{4,16} when the target radius approximates to the wavelength (at 8 MHz the wavelength of sound in water is 187 µm). In these conditions a variety of complex effects are

expected, shown in Fig. 2a. Below this critical region (Fig. 2b) a monotonic relationship was found but its slope is either too flat or too steep for practical use. However, for bubble diameters >300 µm another simple relationship was indicated and has in fact been supported by experiments with glass spheres (0.3–4 mm diameter) as targets (Fig. 3).

These experiments show that bubbles can be detected down to 10 µm diameter and although larger than previously claimed¹⁰ it seems to be an adequate limit, as no evidence was found microscopically for free bubbles smaller than this. Bubble size can be obtained from image size, for bubbles of diameter >300 µm, but below this a complex situation exists where an increase in bubble size may lead to a decrease in image size. One consequence is that bubble sizes cannot be calculated by applying small pressure changes and interpolating bubble size by observing the change in image size and applying Boyle's law. This technique has been used previously¹⁰ and would lead to erroneous conclusions. Preliminary experiments have shown that the method is capable of detecting both fixed and moving gas bubbles in the legs of decompressed, anaesthetised guinea pigs.

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1. Beckman, E. L. & Elliott, D. H. *Proc. Symp. Dysbaric Osteonecrosis* (US Dept of Health, Education and Welfare, Washington DC, 1974).
2. Berghage, T. E. *Undersea Biomed. Res.* **3**, 387–399 (1976).
3. Spencer, M. P., Campbell, S. D., Sealey, J. L., Henry, F. C. & Lindbergh, J. J. *occup. Med.* **11**, 238–244 (1969).
4. Walder, D. N. & Evans, A. *Nature* **252**, 696–697 (1974).
5. Hemmingson, E. A. *Nature* **267**, 141–142 (1977).
6. Gillis, M. F., Peterson, P. L. & Karagianes, M. T. *Nature* **217**, 965–967 (1968).
7. Evans, A., Walder, D. N. & MacMillan, A. J. F. *Nature* **246**, 522–523 (1973).
8. Buckles, R. G. & Knox, C. *Nature* **222**, 771–772 (1969).
9. MacKay, R. S. *Proc. 2nd Symp. Underwater Physiol.* (Publ. 1181) 41 (eds Lambertsens, C. J. & Greenbaum, L. J.) Acad. Sci-natn Res. Council, (Washington D.C. 1963).
10. Rubissow, G. J. & Mackay, R. S. *Ultrasonics* **9**, 225–235 (1971); *Aerospace Med.* **5**, 473–478 (1974).
11. Nishi, R. Y. *XII U.M.S. Workshop* (ed. Pearson, R.) 50–68 (Undersea Medical Society Inc. Bethesda, 1977).
12. Evans, A. *XIII U.M.S. Workshop* (ed. Pearson, R.) 69–80 (Undersea Medical Society Inc. Bethesda, 1977).
13. Walder, D. N. *XIII U.M.S. Workshop* (ed. Pearson, R.) 157 (Undersea Medical Society Inc., Bethesda, 1977).
14. Morse, P. M. *Vibration and Sound*, 2nd edn (McGraw-Hill, New York, 1948).
15. Epstein, P. S. & Plesset, M. S. *J. chem. Phys.* **18**, 1505–1509 (1950).
16. Strutt, J. W. *The Theory of Sound* (Macmillan, London, 1878).

Somatic polymorphism and seed dispersal

SOMATIC seed polymorphism is the production of seeds of different morphologies or behaviour on different parts of the same plant and is a somatic differentiation rather than the result of genetic segregation¹. This phenomenon appears to be confined to a limited number of families of higher plants (for example, Cruciferae, Compositae, Chenopodiaceae, Gramineae). Seed produced within a somatic polymorphism may vary in size (*Xanthium* sp.²), colour (*Atriplex heterosperma* Bunge³) and/or external structure (*Chenopodium album* L.⁴). These variations in morphology are frequently accompanied by differences in germination requirements with the consequence that the germination of polymorphic seeds may be staggered in time. Such somatic channelling of progeny into distinct classes of behaviour may increase the fitness of individuals in environments that are temporally patchy, at the expense of the intrinsic rate of increase that could be attained by a unique seed type in a

uniform environment. It might be expected that somatic seed polymorphism would also be found in species occupying habitats that are patchy in space and under these circumstances polymorphism in dispersal mechanisms could also be anticipated. One reported case may come in this category; *Gymnarrhena micracantha* Desf.⁵, a desert annual, produces numerous small aerial wind-dispersed achenes (the fruit in Compositae is usually termed an achene but is strictly a pseudo-nut as the pericarp is partly axial and there is more than one carpel⁶) and also larger subterranean achenes close to the parent plant. The wind dispersed achenes are produced in greater numbers in years with high rainfall. The subterranean achenes serve only to establish descendants in the safe sites previously occupied by their parents. We report here an even more striking polymorphic dispersal mechanism in the biennial *Picris echioides* L. (Compositae).

P. echioides (bristly ox-tongue) is a fugitive species associated with woodland and wayside clearings. The plants grow to about 50 cm tall and the stems and leaves are covered with numerous bristles. Yellow flower heads are surrounded by an involucre of leafy hairy bracts. Flowers within the capitula are ligulate, the majority producing rufous achenes, transversely wrinkled towards the short beak which bears a pappus of 20 white silky hairs. However, each of the larger marginal outer achenes is tightly held in an enclosing involucre bract; these achenes are covered with short white hairs and have a shorter beak and shorter, vestigial pappus hairs (Fig. 1). Inner achenes weigh about 0.015 g and the dissected embryo weighs 0.005 g. Outer achenes weigh about 0.029 g and the embryo 0.010 g. There are approximately 25 inner and six outer achenes per involucre. The inner achenes are clearly wind dispersed, although the pappus and beak readily break off during or after dispersal. The outer achenes remain within their bracts when the inflorescence falls as a whole. The bracts are hairy and sharply bent, suggestive of animal dispersed propagules.

Three hundred pappus-bearing inner and 300 bract-enclosed outer achenes were scattered randomly on the floor of a 90 cm × 60 cm plastic enclosure. Two laboratory mice (*Mus musculus*) were released in the container for 35 min; the number of seeds transported, the seed retention time and the behaviour of the mice were recorded. In a further experiment a laboratory mouse was placed in a straw-lined 120 cm × 60 cm enclosure, fed on a diet of laboratory pellets and seeds of *Festuca rubra* and allowed to habituate for 7 d, during which time the mouse established a nest. Two hundred and fifty inner achenes and 250 outer achenes (in bracts on senesced flowerheads) were scattered randomly within the enclosure. The transport of achenes to the nest by the mouse was recorded daily. The outer achenes stuck to the forefeet, hindfeet and tail base of the mice in the first experiment; several outer achenes were carried for up to 195 s

Fig. 1 Achenes of *Picris echioides*. Left, inner pappus-bearing achene; right, outer achene enclosed in involucre bract.

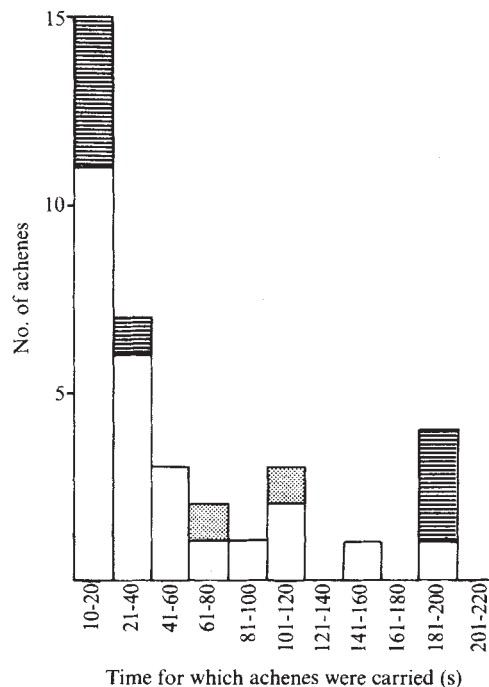
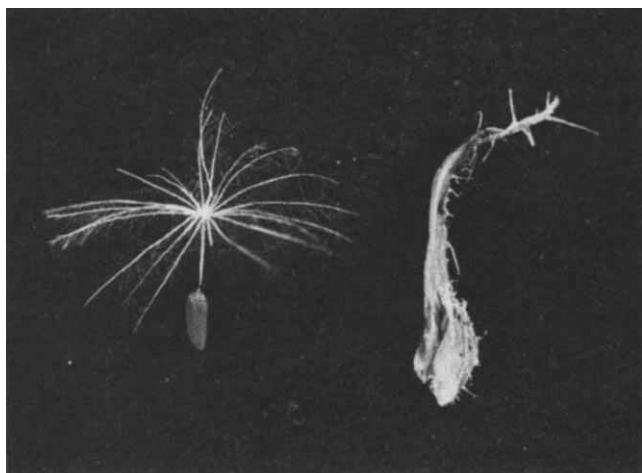


Fig. 2 Frequency distribution of the time for which the outer (bract-enclosed) achenes of *Picris echioides* remained attached to laboratory mice. No inner (pappus-bearing) achenes were ever observed to adhere to the mice. ▨, Attached to tail; □, attached to forefoot; ▩, attached to hindfoot.

(Fig. 2). The adhered achenes did not appear to irritate the mice, nor did they seem to be attractive as a food source. In a few cases the mice actively removed the seeds by chewing but the majority of achenes dropped off passively. No inner achenes were ever observed to adhere to a mouse. A daily count of seeds in the second experiment showed that there was a continued transport of senesced flowerheads (containing outer achenes) to the established nest of the mouse. Again no inner achenes were ever transported in this manner.

The transport of whole capitula implied active caching or the removal of attached flowerheads through grooming at the nest site. The disinterest of mice in outer achenes as a food source in the first experiment and the high chaff to embryo ratio of outer achenes, which must reduce their digestive value, suggests that caching is unlikely. Instead the accumulation of outer achenes in the nest appears to be due to passive transport followed by grooming.

It might be expected that the two types of achene, which differ in size, would have different dormancies, rates of seedling growth or competitive ability (particularly when grown in mixed populations⁷). A series of experiments revealed no such differences⁸. It appears therefore that the achene dimorphism in *Picris echioides* may be related simply to differences in dispersal behaviour. In nature, potential animal dispersers of *P. echioides* include *Clethrionomys glareolus*, *Microtus agrestis*, *Micromys minicticus* and *Apodemus sylvaticus*. The behavioural patterns and nesting habits of these small mammals may well effect transport of the seeds of *P. echioides* into habitats quite different from those that would be reached by wind dispersal.

Somatic seed polymorphism results in the production of a constant ratio of seed types regardless of short term 'patchiness' of the environment and prevents 'tracking' of transient changes. This phenomenon contrasts with the genetic polymorphism of seed dispersal mechanisms such as that found in *Aegilops speltoides* Tausch⁹ in which two kinds of dispersal unit are borne, but on different individuals. Such genetic polymorphism is sensitive to changing direction and intensities of selection, hence the observed fixation of monomorphic populations on the fringes of the distribution area of *A. speltoides*. In contrast, somatic polymorphisms as in *P. echioides*, are buffered against rapid response

to selection; all individual plants are monomorphic and every individual appears to bear polymorphic achenes.

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1. Harper, J. L. *Population Biology of Plants* (Academic, London, 1977).
2. Crocker, W. *Bot. Gaz.* **42**, 265 (1906).
3. Frankton, C. & Bassett, I. J. *Can. J. Bot.* **46**, 1309–1313 (1968).
4. Williams, J. T. & Harper, J. L. *Weed Res.* **5**, 141–150 (1965).
5. Koller, D. & Roth, N. *Am. J. Bot.* **51**, 26–35 (1964).
6. Willis, J. C. *Dictionary of Flowering Plants and Ferns* (Cambridge University Press, 1960).
7. Black, J. N. *Aust. J. agric. Res.* **7**, 98–109 (1956).
8. Sorensen, A. E. thesis, Univ. North Wales (1978).
9. Zohary, D. & Imber, D. *Heredity*, **18**, 223–231 (1963).

Cell sorter analysis of leukaemia-associated antigens on human myeloid precursors

NORMAL and malignant B cells, some monocytes and blast cells from both the common (non-T, non-B) form of acute lymphoblastic leukaemia (ALL) and acute myeloblastic leukaemia (AML) share Ia-like antigenic determinants^{1–8}. ALL cells, but not AML cells, can be further characterised by the expression of an 'ALL-associated' membrane antigen^{9,10}. Although the ALL antigen and the Ia-like antigens on leukaemic blasts have at one time been regarded as candidate leukaemia-specific antigens, it has been suggested that both may be normal differentiation antigens of haematopoietic precursors, their expression in leukaemia reflecting the cellular origins of the disease^{2,7–11}. In immunofluorescent studies combined with phase contrast examination, Ia-like antigens can be demonstrated on normal and leukaemic myeloblasts and some promyelocytes, but not on most promyelocytes and maturing granulocytic or erythroid cells^{7,8}. The presence of Ia-like antigens on myeloid-committed stem cells is supported indirectly by results of functional assays *in vitro* which show the abolition of myeloid colony-forming cells (CFUc) by incubation with complement and hetero-antisera to B cells^{12,13}. Two disadvantages of the approach used in the latter experiments are, first, that the cells of interest are eliminated and no longer available for analysis, and second, that their functional inhibition does not necessarily establish that they carry the antigens in question, as the development of colonies involves stimulatory factors^{14,15} and auxiliary cells,

Table 2 Morphology of separated Ia⁺ and Ia[−] bone marrow cells

	Adult bone marrow			Fetal bone marrow		
	Unsorted	Ia ⁺	Ia [−]	Unsorted	Ia ⁺	Ia [−]
Blast cells	2.5	35.5	2	13	33*	8
Promyelocytes	1	32.5	2	7	10	8
Myelocytes	30	12.5	42	17	2	18
Maturing granulocytic cells	29.5	6	21	11	3	30
Monocytes	7.5	3	3	3	3	4
Red cell precursors	16	2	20	9	0	10
Lymphocytes	13	7.5	10	31	36	14

Cells were prepared as described in Table 1 and separated on FACS. Smears were made and stained with May–Grünwald–Giemsa. Differential counts are shown as percentages.

*Most cells are myeloblasts. Some resemble lymphoblasts.

whose activity, rather than CFUc *per se*, might be compromised by the antibodies used. We now report direct evidence for the presence of the antigens on bone marrow cells by separating them in a fluorescence-activated cell sorter (FACS), according to their binding of anti-Ia-like (anti-p28,33) or anti-ALL antibodies. Functional analysis of the resultant populations reveals that a proportion of human colony and cluster forming cells express the Ia-like hetero-antigen but not the ALL-associated antigen.

For convenience, we refer below to the anti-p28,33 sera as anti-Ia and to the cells bearing the p28,33 determinants as Ia⁺. This terminology presumes more than is proven of the genes involved in the control of these molecules in man; however, the human B cell p28,33 complex has strong structural similarities to rodent Ia molecules^{4,16,17}. The 33K component in man is apparently regulated by genes on chromosome 6 (ref. 18) and carries the polymorphic determinants which are identified by human anti-(HLA) D locus sera^{19,20}. The membrane antigens specifically reactive with anti-p28,33 on leukaemic blasts are, as on B cells, associated with a noncovalently bonded p28,33 complex²¹. The anti-ALL antibodies used here, although produced by immunisation with whole cells, selectively bind to a single glycosylated polypeptide species with an apparent molecular weight of 100,000 (ref. 22). Reactive cells are referred to as ALL⁺.

After elimination of most mature granulocytic and erythroid cells by Ficoll–Trisil density centrifugation, adult marrow samples contained 10–14% cells reactive with anti-Ia serum (Table 1). Ia⁺ cells included larger cells, mainly myeloblasts

Table 1 Characterisation of Ia⁺ cells in human bone marrow with membrane markers

Source of bone marrow	Adult (61 yr)			Fetus (19 weeks)			Regenerating (6 yr) (off treatment for ALL)		
	Total*	Small*	Large*	Total	Small	Large	Total	Small	Large
Cells reacting with:									
Normal rabbit serum	<1			<1			<1		
Anti-Ia-like serum (Ia ⁺)	14	4	10	48	30	18	32	20	12
Anti-ALL serum (ALL) [†]	<1			13	12†	1	14	14†	0
Anti-Ig (SmIg ⁺)	5	4	1	10	10†	0	10	9†	1

To eliminate red cells, normoblasts and granulocytes, cells were separated on Ficoll–Trisil and treated with 0.17 M NH₄Cl for 15 min. The cells were then passed through fetal calf serum and washed. The antisera used were ultracentrifuged before use (100,000g for 1 h). Cells were incubated for 20 min at 4 °C with either normal rabbit serum (NRS 1:10 dilution) or rabbit anti-Ia serum made against purified human p28,33 Ia-like antigens (Ia; 1:20 dilution)^{4,5,8} or rabbit anti-ALL serum (ALL; 1:10 dilution)^{9,21}, washed three times, incubated for 20 min with fluorescein-labelled anti-rabbit immunoglobulin (1:50 dilution) and washed three times again. Other samples were incubated with goat anti-human immunoglobulin directly labelled with fluorescein or rhodamine-isothiocyanate (anti-Ig-FITC or anti-Ig-TRITC) (1:10 dilution) and washed three times. The samples shown were analysed by the FACS (see Fig. 1). Adult bone marrow samples were obtained from ribs removed during operation for lung cancer. Patients were 55–65 yr old. Fetal bone marrow samples were from 19–21-week-old fetuses. Representative experiments are shown. Regenerating marrow samples were obtained from children after the completion of chemotherapy regimes given for the treatment of acute lymphoblastic leukaemia. The bone marrow sample shown was taken from a 6-yr-old child who had been off treatment for 8 months. The marrow showed moderate lymphocytosis. The analysis was carried out 7 months ago and the child is still in complete remission of his leukaemia.

*% of cells showing positive membrane staining under UV microscope.

†Double marker experiments (anti-ALL: FITC; anti-Ig: TRITC) showed that ALL⁺ cells were SmIg[−], and SmIg⁺ cells were ALL[−] (ref. 11). Ia⁺ lymphocytes failed to form rosettes with sheep erythrocytes and did not react with anti-thymocyte serum²².

Table 3 Characterisation of Ia⁺ cells producing granulocyte-macrophage clones *in vitro*

Source of bone marrow	Adult		Fetus		Regenerating (off ALL treatment)	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Cells were incubated with:						
1 None	38.1 ± 7.5		52.5 ± 4.0		211.1 ± 22.2	
2 NRS	32.0 ± 14.3		NT		248.9 ± 15.6*	
3a Anti-Ia serum, unsorted	61.2 ± 6.2		73.1 ± 6.1		272.4 ± 9.3	
3b Sorted Ia ⁺ cells, total	110.7 ± 15.2	15.5(47.3%)	196.1 ± 30.2	94.1(90.5%)	—	
3c Sorted Ia ⁺ cells, small	NT		2.3 ± 1.6		56.0 ± 17.8	11.2(3.2%)
3d Sorted Ia ⁺ cells, large	NT		153.7 ± 11.4		456.4 ± 102.2	54.8(15.8%)
3e Sorted Ia ⁻ cells	20.1 ± 5.7	17.3(52.7%)	19.1 ± 0.8	9.9(9.5%)	413.3 ± 81.4	281.0(81.0%)
Calculated totals after sorting		32.8(100%)		104.0(100%)		347.0(100%)

Preparation of cell suspensions is shown in Table 1. Histograms of the stained cell suspensions were made on the FACS (Fig. 1) and Ia⁺ large and small cells and Ia⁻ cells were separated. Cells in each fraction were counted, plated in double-layer agar cultures¹⁵ and incubated for 7 d. In all experiments, changes in numbers of colonies (>40 cells per clone) corresponded with changes in clusters (<40 cells per clone). In addition, no difference was detected in the proportion of compact (granulocytic) and diffuse (macrophages + mixed) colonies. NT, not tested. *a*, Observed values expressed as numbers (±s.d.) of colonies and clusters (that is, clone-forming cells) per 10⁴ cells plated. *b*, Calculated numbers of Ia⁺ and Ia⁻ clone-forming cells in 10⁴ cells of the original cell population. The formula is: value *a* × proportion of Ia⁺ or Ia⁻ cells (from Table 1). The sum of Ia⁺ and Ia⁻ *b* values shows the total number of clone-forming cells per 10⁴ cells recovered after sorting. If this is taken as an arbitrary 100%, then the relative contribution of Ia⁺ and Ia⁻ cells can be given (in parenthesis).

*These cells were passed through the FACS but were not sorted.

and promyelocytes (Table 2), and smaller SmIg⁺ (surface membrane-immunoglobulin-positive) B lymphocytes. The Ia⁻ cells were myelocytes, maturing granulocytic and erythroid cells, a few lymphocytes and <5% myeloblasts and promyelocytes.

In normal fetal bone marrow and regenerating juvenile marrow, more cells (30–50%) reacted with anti-Ia serum than in adult marrow (Fig. 1*b,c*, Table 1). Again, the Ia⁺ cells included large lymphoblasts, myeloblasts and promyelocytes as well as small lymphocytes. The latter were heterogeneous by membrane marker criteria: 8–15% were B lymphocytes (SmIg⁺, ALL⁻) and the remaining lymphocytes reacted weakly with anti-ALL serum (ALL⁺), were negative for SmIg (SmIg⁻) and failed to form rosettes with sheep erythrocytes or to react with anti-thymocyte serum. These non-T,non-B ALL⁺ cells are not readily detectable in the adult marrow samples^{9,11}. As in the adult marrow, the Ia⁻ cells in fetal and regenerating marrows were mainly maturing granulocytic and erythroid cells; however, Ia⁻ myeloblasts were also seen (Table 2).

The data obtained in the myeloid clonal assays are shown in Table 3. The binding of anti-Ia antibodies to bone marrow cells in the absence of complement (Table 3, line 3a) and manipulations with the cells on the FACS (Table 3, line 2, last column) neither inhibited nor significantly stimulated clone-forming cells. A striking finding was that colony and cluster-forming cells were present in both Ia⁺ and Ia⁻ fractions. Accumulation of

these clone-forming cells in the Ia⁺ fraction (Table 3, line 3b) with concomitant depletion in the Ia⁻ fraction (Table 3, line 3e) was observed in five of seven experiments. Note, however, that in the Ia⁺ fractions invariably fewer cells were observed than in the Ia⁻ fractions (Table 1). When the total contribution of the different cell populations to the colony and cluster-forming cells was calculated in the different experiments, the Ia⁺ cells contained 10–80% of clone-forming cells (mean 40%, seven experiments). Representative values are shown in Table 3. The remaining clone-forming cells (mean 60%) were sorted into the Ia⁻ fractions. The wide diversity of Ia expression on clone-forming cells was confirmed when adult marrow cells were separated into strongly Ia⁺, moderately Ia⁺ and Ia⁻ fractions. Colony and cluster-forming cells were observed in all three populations (Table 4). Mixed and pure neutrophil-granulocyte and macrophage clones and pure eosinophil clusters (8–14%) were found in cultures of all fractions.

Clone-forming cells were found almost exclusively among the intermediate-to-large cells (Table 3, line 3d), suggesting that small Ia⁺, ALL⁺ non-T,non-B cells did not form myeloid colonies. This notion was directly confirmed. Fetal marrow cells were stained with anti-ALL serum, separated on the FACS and cultured for 7 d. The ALL⁺ and ALL⁻ fractions gave, respectively, 3.2 and 626 clones per 10⁴ cells.

We interpret these results to suggest that a proportion of human myeloid precursors are Ia⁺ and a few of them are very

Fig. 1 Bone marrow cells were stained with anti-Ia serum (*a–c*) or normal rabbit serum (*d*, negative control) as described in Table 1. Histograms made on the FACS-1 (Becton Dickinson) are shown. The cells were separated on the basis of fluorescence intensity (Ia⁺ compared with Ia⁻ fractions). Ia positivity was arbitrarily defined as staining intensity above negative controls (*d*). The nonspecific staining in suspensions incubated with NRS was checked on the UV microscope in each experiment and found to be minimal. Some samples were also separated on the basis of cell size (small against large) as indicated. The cells within the shaded areas were discarded. The standard conditions of FACS setting were: laser power 400 mW at 488 nm; scatter channel gain 2; fluorescence channel gain 8; photomultiplier tube 700 V (see footnote to Table 4). In these conditions the proportions of Ia⁺ and Ia⁻ cells detected on the FACS agreed with the observations made on the UV microscope (Table 1). *a*, Adult; *b*, fetal; *c*, regenerating.

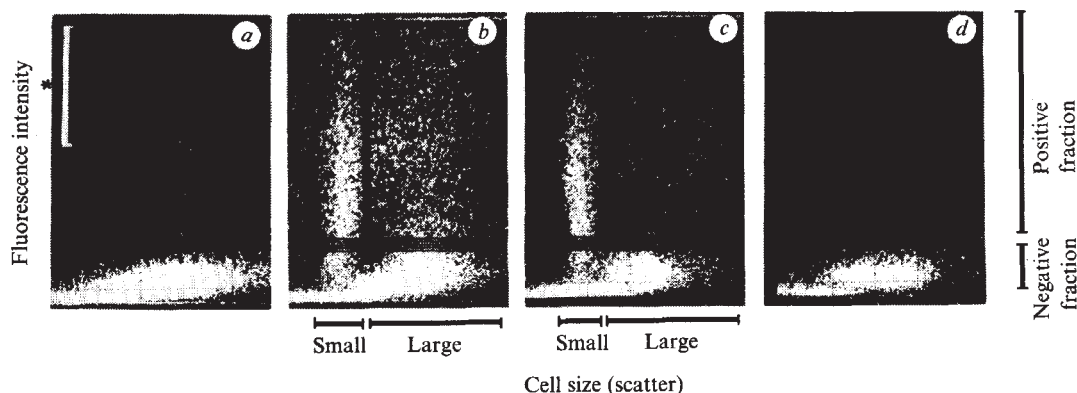


Table 4 Colony and cluster-forming cells express a wide range of Ia densities on the cell membrane

Strong Ia ⁺	581 ± 172*
Moderately Ia ⁺	449 ± 24
Ia ⁻	217 ± 44
Unseparated	277 ± 57

Normal adult bone marrow cells were stained with anti-Ia serum and separated into strongly Ia⁺, moderately Ia⁺ and Ia⁻ fractions on the FACS and cultured (clones per 10⁴ cells plated ± s.d.).

*Intensity of fluorescent staining is shown in Fig. 1a by an asterisk.

strongly Ia⁺. As very weakly Ia⁺ cells might have been sorted into the Ia⁻ fraction in the conditions used on the FACS, our results do not exclude that a larger proportion of clone-forming cells carry Ia-like antigens when analysed by other techniques, for example, by complement-mediated cytotoxicity. Nevertheless, our data indicate a striking heterogeneity of Ia expression on myeloid precursors which could not be appropriately documented with other techniques. The results also strongly suggest that small cells of lymphoid morphology with the antigenic phenotype of acute lymphoblastic leukaemia (ALL⁺, Ia⁺) are not CFUc. This fact has not been unequivocally established, as ALL⁺ cells might require the collaboration of large Ia⁺ cells for their development into colonies. This possibility seems unlikely, however, as in our experiments the separated cells were cultured on peripheral blood feeder layers which provided an ample source of colony-stimulating factors²³.

These findings suggest that the two distinct Ia⁺ non-T, non-B cell types (that is, the smaller ALL⁺ cell and the larger ALL⁻ cell which express some myeloid characteristics) might represent the target cells for leukaemic transformation in the common (non-T, non-B) form of ALL and in AML respectively. This implies that leukaemic blasts largely retain at least some of the antigenic characteristics of cells from which they are derived¹¹. Alternatively, the target cell in ALL and AML could be an even less mature haematopoietic stem cell, subsequent maturation of the leukaemic clones being 'frozen' at the level corresponding to the two cell types. The nature of the ALL⁺, Ia⁺ 'lymphoid' cells is unknown; we regard these cells as candidate lymphocyte precursors or stem cells¹¹.

These and previous studies^{7,8,24,25} indicate a maturation-linked expression of Ia-like antigens with a progressive loss during granulocyte differentiation. Whether the striking variability of Ia-like antigen expression on CFUc cells is related to particular stages of cell differentiation, governed by events linked to the DNA synthetic cell cycle^{25,26} or due to other factors is essentially unknown and warrants further study.

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- Humphreys, R. E. *et al. J. exp. Med.* **144**, 98–112 (1976).
- Schlossman, S. F., Chess, L., Humphreys, R. E. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1288–1299 (1976).

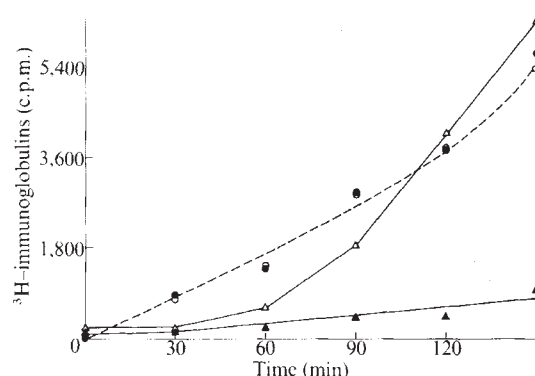
- Billing, R. *et al. J. exp. Med.* **144**, 167–178 (1976).
- Snary, D., Barnstable, C. H., Bodmer, W. F., Goodfellow, P. N. & Crumpton, M. J. *Scand. J. Immun.* **6**, 439–452 (1977).
- Welsh, K. I. & Turner, M. J. *Tissue Antigens* **8**, 197–205 (1976).
- Winchester, R. J. *et al. J. exp. Med.* **141**, 924–929 (1975).
- Winchester, R. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4012–4016 (1977).
- Janossy, G. *et al. Br. J. Haemat.* **37**, 391–402 (1977).
- Brown, G., Hogg, N. & Greaves, M. F. *Nature* **258**, 454–456 (1975).
- Greaves, M. F. *et al. in Immunological Diagnosis of Leukaemias and Lymphomas* (eds Thierfelder, S., Rodt, H. & Thiel, E.) 61–75 (Springer, Berlin, 1977).
- Greaves, M. F., Janossy, G., Francis, G. E. & Minowada, J. in *Cell Proliferation*, Vol. 5 (eds Clarkson, B., Till, J. & Marks, P.) (Cold Spring Harb. Press, in the press).
- Cline, M. J. & Billing, R. J. *exp. Med.* **146**, 1143–1145 (1977).
- Kaplan, J., Inoue, S. & Offenbreit, M. J. *Nature* **271**, 458–459 (1978).
- Metcalfe, D. & Foster, R. *Proc. Soc. exp. Biol. Med.* **126**, 758–762 (1967).
- Paran, M. & Sachs, L. *J. Cell Physiol.* **72**, 247–249 (1968).
- Springer, T. A., Kaufman, J. F., Terhorst, C. & Strominger, J. L. *Nature* **268**, 213–218 (1977).
- Springer, T. A. *et al. Cold Spring Harb. Symp. quant. Biol.* **41**, 387–396 (1976).
- Barnstable, C. J. *et al. Cold Spring Harb. Symp. quant. Biol.* **41**, 443–456 (1976).
- Giphart, M. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3533–3536 (1977).
- Snary, D., Barnstable, C., Bodmer, W. F., Goodfellow, P. & Crumpton, M. J. *Cold Spring Harb. Symp. quant. Biol.* **41**, 379–386 (1976).
- Sutherland, D. R., Smart, J. & Greaves, M. F. *Leuk. Res.* **2**, 115–126 (1978).
- Greaves, M. F. & Janossy, G. *In Vitro Methods in Cell Mediated Tumour Immunity* (eds Bloom, B. R. & David, J. R.) 89–111 (Academic, New York, 1976).
- Pike, B. L. & Robinson, W. A. *J. cell. comp. Physiol.* **76**, 77–84 (1970).
- Janossy, G., Greaves, M. F., Capellaro, D., Roberts, M. & Goldstone, A. H. *Immunological Diagnosis of Leukaemias and Lymphomas* (eds Thierfelder, S., Rodt, H. & Thiel, E.) 97–107 (Springer, Berlin, 1977).
- Ross, G. D., Jarowski, C. L., Rabellino, E. M. & Winchester, R. J. *J. exp. Med.* **147**, 730–744 (1978).
- Jerry, L. M. *et al. Cancer Res.* **36**, 3446–3452 (1976).
- Ferrone, S. *et al. in Protides of Biological Fluids, 25th Symp.* (ed. Peeters, H.) 645–655 (Pergamon, Oxford, 1977).

Novel form of T-cell stimulation of immunoglobulin secretion

THE precise mechanism of action by which helper T cells induce precursor B cells to acquire the capacity to secrete immunoglobulins^{1,2} remains poorly understood. This paucity of information is due, in part, to the temporal lag between the initial T cell–B cell interactions relatively early in the process and the subsequent expression of these interactions a day or more later^{3,4}. This time lag presumably relates to the time required for B cells to undergo a maturation or differentiation after interaction with T cells. We now report that, using a pulse-label technique, we have observed that the addition of thymus cells to previously cultured spleen populations during the 90-min pulse-label period resulted in a marked increase in the rate of secretion of immunoglobulin. Moreover, the enhancement occurred without detectable delay.

Unimmunised New Zealand white rabbits were used as a source of lymphoid tissue. The method for preparing cell

Fig. 1 Effect of thymocytes on the rate of appearance of intracellular and extracellular ³H-immunoglobulin. The procedure is described in the legend to Table 1. Spleen cells were cultured for 72 h before use and thymocytes were isolated on the day of use. ▲, △, Extracellular fluid; ●, ○, intracellular fluid. Open symbols, both splenocytes and thymocytes; closed symbols, splenocytes only.



suspensions⁵ and the procedure used for tissue culture were as previously described^{6,7}. Assessment of immunoglobulin production by coprecipitation analysis⁸ and the preparation and specificity characteristics of anti-thymocyte serum has also been described^{7,9}. Spleen cells were centrifuged on a Ficoll gradient and those cells concentrated atop the 30% layer were used in cell culture¹⁰.

The effect of mixing separately cultured splenocytes and thymocytes is shown in Table 1. Although freshly isolated spleen cells failed to exhibit increased ³H-immunoglobulin secretion on addition of thymocytes (experiment 1), spleen and thymus cells, cultured separately for 2 or 3 days before being mixed at the beginning of the 90-min pulse-label period, yielded increased ³H-immunoglobulin secretion above that observed with spleen alone.

From the results of experiment 1 it was not clear whether both thymus and spleen cells required a prior culture period before being mixed in the pulse-label assay. Accordingly, an experiment was performed in which the duration of prior incubation for each cell type was varied. It can be seen (Table 1, experiment 2) that it was necessary to culture spleen cells for at least 2 days and preferably for 3 days in order to observe the rapid onset effect of thymus cells. In contrast, prior incubation of thymus cells was not required and, indeed, tended to reduce the extent of stimulation that was ordinarily observed with fresh thymus cells.

Table 1 Effect of mixing separately cultured splenocytes and thymocytes

Expt no.	Cells present during pulse-label period	Length of prior incubation of:		³ H-immunoglobulin secreted during pulse-label period (c.p.m.)
		spleen (d)	thymus (d)	
1	Spleen	0	NA*	60
	Spleen + thymus	0	0	40
	Spleen	1	NA	90
	Spleen + thymus	1	1	80
	Spleen	2	NA	360
	Spleen + thymus	2	2	680
	Spleen	3	NA	720
	Spleen + thymus	3	3	2,570
2	Spleen	3	NA	230
	Spleen + thymus	3	3	1,530
	Spleen + thymus	3	2	3,450
	Spleen + thymus	3	1	3,210
	Spleen + thymus	3	0	4,970
	Spleen	2	NA	90
	Spleen + thymus	2	3	200
	Spleen + thymus	2	2	360
	Spleen + thymus	2	1	510
	Spleen + thymus	2	0	750
	Spleen	1	NA	50
	Spleen + thymus	1	3	60
	Spleen + thymus	1	2	80
	Spleen + thymus	1	1	70
	Spleen + thymus	1	0	100

Spleen and thymus cells were separately cultured in Medium 199 containing 10% fetal calf serum for the indicated number of days. After being washed with leucine-free medium 199, spleen cells (10^7) with or without thymus cells (6×10^7) were incubated in the presence of such medium containing ³H-leucine for 90 min at 37°C. At the end of this period, the ³H-immunoglobulins secreted into the extracellular fluids were assessed by coprecipitation of one aliquot in the presence of carrier rabbit immunoglobulin and goat anti-rabbit immunoglobulin and another aliquot in the presence of egg albumin and goat anti-egg albumin. The difference in these samples reflects specifically precipitable rabbit immunoglobulins. The values shown for mixtures of spleen and thymus have been corrected for the small amounts of ³H-immunoglobulin detected when thymus cells were incubated alone. This correction corresponded to 1% of the ³H-immunoglobulins found when spleen and thymus cells were incubated together.

* NA, not applicable.

Table 2 Evidence for T-cell participation in enhancement of secretion of immunoglobulin

Cells added to spleen cells	³ H-immunoglobulin secreted (c.p.m.)
None	530
Thymus	2,200
Thymus (ATS*-treated)	740
None	400
T-cells (nylon wool)	2,300
Thymus	2,600

The procedure is described in the legend to Table 1. Spleen cells were cultured for 72 h before use, whereas the thymus cells used were freshly isolated. Spleen T-cell enriched populations were prepared on nylon wool columns¹³ using freshly isolated cells.

* ATS, antithymocyte serum.

Analysis of the effect of thymocytes on the rate of appearance of intra- and extracellular ³H-immunoglobulins revealed that the rate of appearance of intracellular ³H-immunoglobulins within spleen cells was essentially unaltered by the presence of thymus cells, whereas thymocytes markedly enhanced the rate of appearance of extracellular immunoglobulin (Fig. 1). However, meaningful comparison of the rate of appearance of intracellular and extracellular immunoglobulins may not be possible because intracellular ³H-immunoglobulins are composed not only of molecules destined for secretion but also of molecules that are sequestered, for example, surface immunoglobulins. Furthermore, since the proportion of cells containing intracellular ³H-immunoglobulins which respond to thymus cells is not known, the curves for the intra- and extracellular appearance of ³H-immunoglobulins may not be directly related.

In other experiments using shorter time intervals, the initial thymocyte-induced enhancement of ³H-immunoglobulin secretion was detectable at 20 min (data not shown). The precise time of onset of enhanced ³H-immunoglobulin secretion due to thymocytes cannot be closely defined for a period earlier than 15–20 min because lymphoid cells exhibit a secretory lag of approximately 15 min^{11,12} during which little or none of the readily detectable intracellular ³H-immunoglobulins can be observed extracellularly. Considered in this context there was essentially no delay in the onset of enhanced secretion due to thymus cells.

The effect of thymocytes was specific in that the rate of extracellular appearance of ³H-labelled serum albumin from liver cells was not enhanced when liver cells were incubated in the presence of thymus cells. Evidence consistent with the view that T cells participate in the rapid onset enhancement of immunoglobulin secretion is shown in Table 2. Thus, the stimulation by thymocytes was lost by prior treatment with antithymocyte serum and complement. In addition, freshly isolated splenic T-cell enriched populations¹³ were as efficacious as thymocytes.

Of the ³H-immunoglobulins secreted in response to thymus cells approximately 65% were accountable for as IgM and 35% as IgA by specific coprecipitation analysis. In contrast, ³H-immunoglobulins of the IgG class were not detected. The foregoing experiments have been performed using spleen cells cultured in the absence of added antigen and the resulting immunoglobulin secretion measured by a pulse-label assay. Recently, we have been able to detect the same rapid onset enhancement due to thymus cells using spleen cells cultured with sheep red blood cells (RBC) as antigen and a plaque assay for sheep RBC.

It will be of considerable interest to determine whether the secretagogue-like activity of thymocytes induces a substantial change in B cells or a subtle change. For example, does the alteration require the assembly or reinforcement of most of the cells' total secretory apparatus or does it simply alter the rate of

release of completed molecules awaiting discharge at some terminal compartment very close to the end of the intracellular translocation process?

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1. Miller, J. F. A. P. & Mitchell, G. F. *Transplant. Rev.* **1**, 3-42 (1969).
2. Claman, H. N. & Chaperson, E. A. *Transplant. Rev.* **1**, 92-113 (1969).
3. Stavitsky, A. B. & Cook, R. G. *J. Immun.* **112**, 583-616 (1974).
4. Zimmerman, D. H., Okumura, K., Rabkin, C. & Kern, M. *J. Immun.* **113**, 1891-1896 (1974).
5. Swenson, R. M. & Kern, M. *Proc. natn. Acad. Sci. U.S.A.* **57**, 417-422 (1967).
6. Zimmerman, D. H. & Kern, M. *J. Immun.* **111**, 761-769 (1973).
7. Zimmerman, D. H. & Kern, M. *J. Immun.* **111**, 1326-1333 (1973).
8. Swenson, R. M. & Kern, M. *Proc. natn. Acad. Sci. U.S.A.* **59**, 546-553 (1968).
9. Okumura, K. & Kern, M. *Cell. Immun.* **17**, 19-29 (1975).
10. Okumura, K., Shinohara, N. & Kern, M. *J. Immun.* **113**, 2027-2034 (1974).
11. Helmreich, E., Kern, M. & Eisen, H. N. *J. biol. Chem.* **236**, 464-473 (1961).
12. Kern, M. in *Glycoproteins of Blood Cells and Plasma* (eds Jamieson, G. A. & Greenwalt, T. J.) 190-203 (Lippincott, Philadelphia, 1971).
13. Trizio, D. & Cudkowicz, G. *J. Immun.* **113**, 1093-1097 (1974).

Absence of H-Y antigen in XY females with dysgenetic gonads

WITH a few exceptions¹⁻⁶, the mammalian testis is known to differentiate in the presence of a Y chromosome⁷, but the mechanism(s) involved remains unknown. Increasing experimental evidence shows that the testis-determining gene product is a plasma membrane protein known as H-Y antigen⁸. Several investigators have demonstrated that the gene(s) controlling H-Y antigen expression are located on the Y chromosome^{9,10}. We now report the absence of H-Y antigen in 46 XY pure gonadal dysgenesis (PGD) in which patients have female internal and external genitalia and 'streak' gonads.

We studied nine subjects (20-38 yr old), three normal males, three normal females and three phenotypic females with primary amenorrhoea. Before treatment the three patients had the limb proportions of eunuchs, and had no breast development or axillary hair, although a few coarse hairs were present on the labia majora. There were no signs of Turner's syndrome. These three patients could therefore be categorised under PGD. Two of them were siblings.

Cytological studies using fluorescent and G-banding techniques on all the three patients confirmed their 46 XY karyotype (Table 1). H-Y antigen was assayed immunologically by the cytotoxicity test¹¹ (Fig. 1). We found that cells from PGD, like those from normal females, did not express H-Y antigen.

There are reports of the familial occurrence of this condition consistent with X-linked inheritance¹². The locus responsible could be the same as that involved in the XY female wood lemmings¹³.

There are three possible explanations of our observations as well as those from XY female wood lemmings: (1) inhibition of

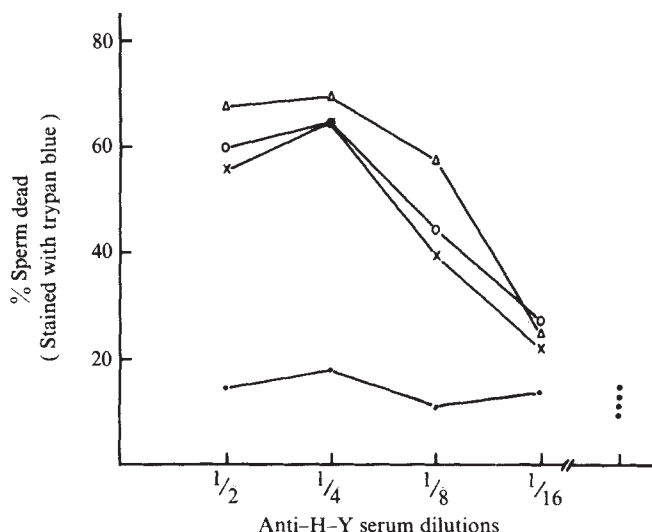


Fig. 1 Cytotoxicity test on mouse sperm with mouse H-Y antiserum after absorption with lymphocytes from three patients with PGD, three normal males and three normal females. Control (C) includes complement but no H-Y antiserum. Each point represents an average value for three separate tests. H-Y antisera were raised in C57 BL/6 female mice by five weekly intraperitoneal injections of spleen cells (4.5×10^6) from C57 BL/6 male mice. Absorption was accomplished by suspending a fixed number of lymphocytes (10^7 cells ml^{-1}) in small tubes containing 0.15 ml of H-Y antiserum diluted one half, and by leaving the suspension for 40 min on ice. Suspensions were agitated gently every 10 min. After absorption, the tubes were centrifuged into aliquots for use in the cytotoxicity test. Cytotoxicity was demonstrated with C57 BL/6 epididymal sperm according to the method of Goldberg *et al.*¹¹. Equal volumes (0.05 ml) of H-Y antiserum (serially diluted 1/2 to 1/16), sperm (5×10^6 cells ml^{-1}) and absorbed rabbit serum (complement source diluted 1/4) were incubated at 37°C for 50 min. Trypan blue dye (0.1 ml) was added during the last 10 min of incubation to stain dead sperm, and cells were counted in a haemocytometer. The failure of loss of cytotoxicity after absorption with female and PGD cells indicates that these cells do not contain H-T antigen. Δ, Unabsorbed antiserum; ○, absorbed, female; ×, absorbed, PGD; ●, absorbed, male.

the testis-determining gene on the Y chromosome by abnormal gene(s) carried either on the X chromosome or on an autosome(s)¹⁴; (2) a mutation at the H-Y locus of the Y chromosome probably inactivating the gene product so that its essential function(s) is affected; (3) an X-linked Y suppressor gene that blocks the expression of the H-Y antigen¹⁵. Whatever the mechanism, it is certain that in the absence of H-Y antigen, as in our cases of PGD, the gonad could not be differentiated into testis. Since Mullerian duct derivatives such as uterus, fallopian tubes and vagina are present in all our patients, it is highly probable that blocking of the expression of H-Y antigen has occurred before initiation of gonadal differentiation.

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1. Cleveland, W. W. & Chang, G. C. *Pediatrics* **36**, 892-898 (1965).
2. Cattanach, B. M., Pollard, C. E. & Hawkes, S. G. *Cytogenetics* **10**, 318-337 (1971).
3. DeLa Chapelle, A. *Am. J. hum. Genet.* **24**, 71-105 (1972).
4. Wachtel, S. S. *et al. New Engl. J. Med.* **295**, 750-754 (1976).
5. Saenger, P. *et al. J. clin. Endocr. Metabol.* **43**, 1234-1239 (1976).
6. Hamerton, J. L. *et al. J. Reprod. Fert. Suppl.* **7**, 25-51 (1969).
7. Welshons, W. J. & Russell, L. B. *Proc. natn. Acad. Sci. U.S.A.* **45**, 560-566 (1959).
8. Wachtel, S. S., Ohno, S., Koo, G. C. & Boyse, E. A. *Nature* **257**, 235-236 (1975).
9. Bennett, D. *et al. Nature* **257**, 236-238 (1975).

Table 1 Cytological status of nine subjects

Diagnosis	Age range (yr)	X chromatin	Y chromatin	Karyotype from blood	No. of metaphase plates scored
Normal male (n = 3)	25-35	-	+	46 XY	75
Normal female (n = 3)	25-38	+	-	46 XX	65
PGD					
S.M.	28	-	+	46 XY	70
N.B.	36	-	+	46 XY	75
P.B.	20	-	+	46 XY	70

N.B. and P.B. are sibs.

10. Wachtel, S. S. *et al.* *New Engl. J. Med.* **293**, 1070-1072 (1975).
11. Goldberg, E. H., Boyse, E. A., Bennett, D., Scheid, M. & Carswell, E. *Nature* **232**, 478-480 (1971).
12. Espiner, E. A., Veale, A. M. O., Sands, V. E. & Fitzgerald, P. H. *New Engl. J. Med.* **283**, 6-11 (1970).
13. Mittwoch, U. *J. med. Genet.* **14**, 335-338 (1977).
14. Sternberg, W. H., Barclay, D. L. & Koplefer, H. W. *New Engl. J. Med.* **278**, 695-700 (1968).
15. Wachtel, S. S. *et al.* *Nature* **264**, 638-639 (1976).

H-2 restriction and non-restriction of T-cell-mediated cytotoxicity against mouse mammary tumour targets

CELL-MEDIATED CYTOTOXICITY (CMC) against C3H/Umc (C3H) syngeneic mammary tumours measured *in vitro*, after *in vivo* immunisation, is mediated by cytotoxic T lymphocytes (CTL) and is directed mostly against cross-reacting antigens related to the mouse mammary tumour virus (MTV)¹⁻³. The long-term CMC assay required for detecting this response against adherent target cells has complex kinetics and at least two superimposing components: (1) an initial early peak of cytotoxicity, detectable at approximately 6 h and accounting for 20-30% of the CMC, and (2) a later peak, detectable after 18-20 h and representing an additional 40-50% of the cytotoxic effect³. Both peaks are mediated by CTL with the Ly23 phenotype (that is, Ly1⁺Ly2,3⁺); however, non-cytotoxic T cells of Ly1 and probably Ly123 phenotype (that is, Ly1⁺Ly2,3⁻ and Ly1⁺Ly2,3⁺, see refs 3 and 4 for nomenclature and functional characteristics of these T-cell subsets) are required for the

Fig. 1 Cell-mediated cytotoxicity (CMC) of lymph node (LN) cells from C3Hf mice (C3H/Umc foster-nursed on C57B1 females, see ref. 1 for details) immunised with C3H/Umc (C3H) mammary tumour cells against syngeneic and allogeneic mammary tumour target cells, shows absolute H-2 restriction of the early CMC peak (2-8 h) and relative H-2 restriction of the late CMC peak (22-26 h). All tumours induced by the mouse mammary tumour virus (M-MTV) have appeared spontaneously and are kept *in vitro* using techniques described in ref. 1. Tumours tested were: C3H/Umc (MT.1 used for immunisation), C3H/HeJ (both have the *H-2 k k* haplotype similar to the LN cell donors), A/J (*H-2 kd*), RIII (*H-2 rr*) and (I $\times$ C57B1/6)F₁ (*H-2 j j/bb*). The immune C3Hf LN cells were obtained from mice immunised by temporary growth in the footpad of a C3H/Umc mammary tumour (MT.1), followed by amputation 15-20 d later and collection of the regional LN 7-9 d after amputation³. Immune LN cells were tested for CMC using ³H-proline-pre-labelled adherent target cells (1,000 cells per well of microtest plates II, Falcon Plastics) and 100:1 effector:target ratios. The mixtures were incubated for the indicated times at 37°C in humidified 5% CO₂ in air and the per cent cytotoxicity calculated as described in ref. 3 in relation to the target cells incubated with non-immune C3Hf LN cells (based on the measurement of the remaining adherent target cells per well after washing in experimental and control groups). For further details on cell preparation, target labelling and recovery of labelled adherent cells, see ref. 3. ●, C3H; ○, C3H/He; △, A; ▲, RIII; □, (I $\times$ C57BL/6)F₁.

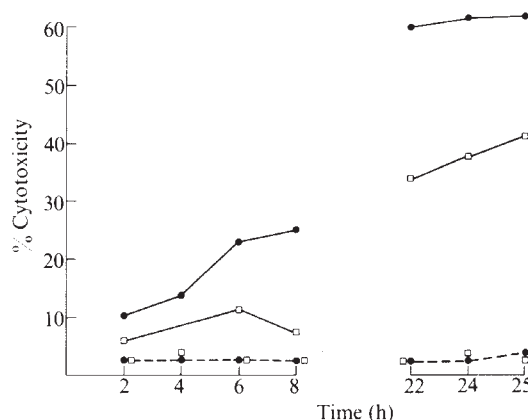
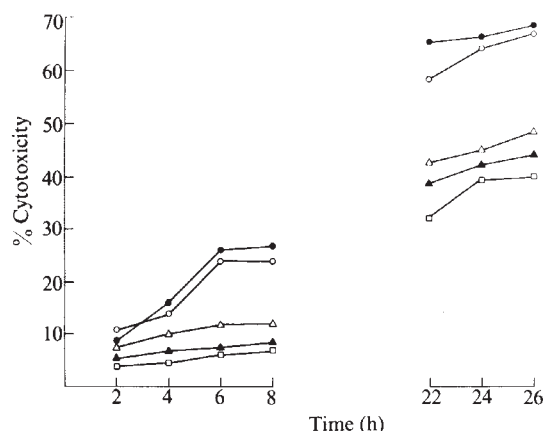


Fig. 2 Abrogation of early and late CMC of specifically immune C3Hf LN cells against syngeneic and allogeneic mammary tumour targets, by pretreatment of the effector LN cells with anti-Ly-2.1 and rabbit complement (C). Ly2 is an antigen expressed on T cells^{3,4}. For details on the CMC assay using ³H-proline-labelled adherent target cells, *in vivo* immunisation and *in vitro* treatment of effector cells with anti-Ly sera, see ref. 3. Treatment with anti-Thy-1.2 and C also produced abrogation of both CMC peaks (data not shown, see also ref. 1). Conversely, removal of B cells by pretreatment with rabbit anti-mouse Ig and C had no effect on CMC; thus, at 24 h CMC was 59% in the syngeneic and 40% in the allogeneic target. Similarly, the addition of rabbit anti-mouse Ig (or control rabbit serum) at concentrations of 100 and 200 µg per culture had no effect on the early or late CMC peaks on the syngeneic or allogeneic target (data not shown). The addition of heat-aggregated goat IgG (at a concentration of 50 or 100 µg per ml of culture media) also had no effect on the early or late CMC peaks (data not shown). These additional studies confirm the view that both CMC peaks on syngeneic or allogeneic targets are mediated by T-effector cells. ●, C3H; □, (I $\times$ C57BL/6)F₁; —, control; ---, anti-Ly-2.1 + C.

expression of the second cytotoxic peak³. On the other hand, the short-term assays of CTL-mediated CMC show single-hit kinetics⁵, perhaps comparable to part of the early CMC peak in our system. During our studies of immunity to syngeneic mammary tumours, we observed that when C3H or C3Hf immune CTL were tested against allogeneic MTV-induced mammary tumour targets, a relative degree of H-2 restriction of CMC was observed², although not of the magnitude detected in other systems using mostly short-term CMC assays⁶⁻¹¹. The experiments reported here show that when the kinetics of the CMC response against adherent syngeneic and allogeneic mammary tumour targets were compared, some differences became apparent: whereas the early CMC peak showed absolute H-2 restriction, no restriction was observed in the late CMC peak, although both events were mediated by Ly23 CTL (ref. 3).

The early CMC peaks (2-8 h, Fig. 1) produced by C3Hf immune CTL against syngeneic C3H or H-2-identical (C3H/He) targets were comparable, whereas CMC was markedly reduced against the allogeneic targets. The late peak (22-26 h, Fig. 1) also seemed to show some degree of H-2 restriction². Figure 2 shows comparable data for the CMC response against C3H and (I $\times$ C57BL/6)F₁ targets during the early and late peaks, but also includes the abrogation of such responses by pretreatment of the CTL with Ly-2.1 serum and C, confirming our previous observation that both CMC peaks are mediated exclusively by Ly23 CTL (ref. 3). Figure 3 shows that when the whole time course of the response of C3Hf CTL against syngeneic or allogeneic mammary tumour targets was measured, it was evident that the late peak, appearing at 18 h, was quite comparable in magnitude and kinetics between the allogeneic and syngeneic targets. Thus, the relative H-2 restriction observed in the late peak in Fig. 1 was due to the absence of the early H-2-restricted CMC peak. It can be concluded that although the early CMC peak in the present model system shows absolute H-2 restriction, no such restriction is observed for the late CMC peak.

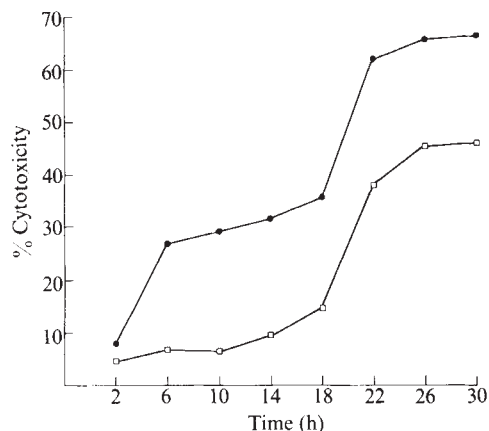


Fig. 3 Time course of CMC of C3Hf specifically immune LN cells against syngeneic (●) and allogeneic (□) mammary tumour target cells, showing absolute H-2 restriction of the early CMC peak (2–18 h) and no H-2 restriction of the late CMC peak (22–30 h), as the shape and magnitude of the late curve are comparable for the syngeneic and allogeneic targets. The difference in total CMC at 30 h between the allogeneic and syngeneic targets which appears as relative H-2 restriction is due to the absence of the early CMC peak in the allogeneic combination. For details on immunisation and assay system see Fig. 1 and ref. 3.

The interpretation of the present results requires some definitions concerning the assay and the system³ in relation to the H-2 restriction model^{6–8}. CTL generated *in vivo* in the local lymph node after hyperstimulation (temporary syngeneic tumour growth and removal) show specific *in vitro* CMC directed against syngeneic (or allogeneic) mammary tumour targets that requires relatively long-term assays for detection^{1–3}. The kinetics of this assay indicate that complex events operate in the generation of the CMC response and that non-cytotoxic helper or amplifier T cells are required for the expression of maximal CMC³. Based on the timing of the response it is probable that the second peak of activity represents tertiary stimulation of memory cells into CTL, as observed in syngeneic and allogeneic systems^{12,13}, whereas the initial peak may represent CMC comparable to the short-term assays in other systems, and a possible measure of conventional CTL activity⁵.

If this sketchy interpretation is accepted, several hypotheses may explain the observed non-restriction of the late peak. (1) Ly1 helper cells. As Ly1 helper-amplifier cells are required for the generation of the non-restricted second peak³, it is possible that specific Ly1 help against the H-2-MTV complex in the allogeneic targets is generated during the long-term assay and that such help can by-pass the H-2-restricted activity of the Ly23 CTL. (2) Memory is non-restricted. Most of the literature on responses to viral and other antigens does not support this hypothesis^{12–17}. In general, syngeneic cells bearing the appropriate antigen can induce good levels of CTL regeneration in secondary or tertiary stimulation^{12–14}, although H-2 identity may not be an absolute requirement^{15–16}. However, it is accepted that the pattern of CMC of secondary cells is similar to that of the primary CTL^{12–17}. (3) Changes in avidity of CTL. In allogeneic systems, it has been shown that avidity of CTL is influenced by time and antigen dose, in terms of antigen-driven selection of pre-cytotoxic T-cell clones with a spectrum of affinities for antigen¹⁸. Thus, cells with high-affinity receptors would be stimulated preferentially by antigen, giving rise early in the response to a low number of CTL with high affinity (and susceptible to H-2 restriction?). Later, cells with lower affinity would be stimulated, especially when the amount of antigen is not limiting, generating CTL with less avidity (and perhaps without H-2 restriction). Some of the kinetic data of the allogeneic responses fit well with the timing described in our assay for the generation of the second CMC peak¹⁸, the putatively less avid, non-H-2-restricted tertiary CTL response. (4) Genetics of restriction. Cross-reactive lysis of hapten-modified

targets has been observed when certain mouse strain combinations are tested¹⁹. One possible explanation of our data could be that secondary or tertiary activation of CTL in our system may show cross-reactive lysis (perhaps also under genetic control).

Several other explanations may account for the observed differences. These could include: (1) intrinsic properties of the assays (that is, most of the H-2 restriction has been shown with short-term assays, and in fact, no restriction was observed when longer assays were used^{15,20–23}); (2) properties of the adherent mammary tumour lines or changes in H-2 expression during the assay (an inverse relationship between expression of MTV antigens and D-end specificities of H-2 has been observed¹); (3) part of the CMC in our system is not mediated by T cells (this point has been eliminated by the experiments described in Fig. 2); (4) our system belongs to a non-restricted category^{20–23}. Experiments to decide between some of these interpretations are in progress.

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1. Stutman, O. *Cancer Res.* **36**, 739–747 (1976).
2. Stutman, O. *Transplant. Proc.* **9**, 1153–1155 (1977).
3. Stutman, O., Shen, F. W. & Boyse, E. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5667–5671 (1977).
4. Boyse, E. A., Cantor, H., Shen, F. W. & McKenzie, J. F. C. *Immunogenetics* **5**, 189 (1977).
5. Cerottini, J. C. & Brunner, K. T. *Adv. Immun.* **18**, 67–132 (1974).
6. Zinkernagel, R. M. & Doherty, P. C. *Contemporary Topics Immunobiol.* **7**, 179–220 (1977).
7. Shearer, G. M., Schmitt-Verhulst, A. M. & Rehn, T. G. *Contemporary Topics Immunobiol.* **7**, 221–243 (1977).
8. Bevan, M. J. *Cold Spring Harb. Symp. quant. Biol.* **41**, 519–527 (1977).
9. Gornard, E., Duprez, V., Henin, Y. & Levy, J. P. *Nature* **260**, 707–709 (1976).
10. Blank, K. J., Freedman, H. A. & Lilly, F. *Nature* **260**, 250–252 (1976).
11. Trinchieri, G., Aden, D. P. & Knowles, B. B. *Nature* **261**, 312–314 (1967).
12. Plata, F. & Jongeneel, V. C. *J. Immun.* **119**, 623–629 (1977).
13. MacDonald, H. R., Sordat, B., Cerottini, J. C. & Brunner, K. T. *J. exp. Med.* **142**, 622–636 (1975).
14. Wettstein, P. J. & Frelinger, J. A. *J. exp. Med.* **146**, 1356–1366 (1977).
15. Herberman, R. B. *et al. J. natn. Cancer Inst.* **53**, 1103–1111 (1974).
16. Senik, A., Gisselbrecht, S. & Levy, J. P. *Int. J. Cancer* **16**, 960–970 (1975).
17. Pang, T., Andrew, M. E., Melvold, R. W. & Blandin, R. V. *Aust. J. exp. Biol. med. Sci.* **55**, 39–48 (1977).
18. Thorn, R. M. & Henney, C. S. *J. Immun.* **119**, 973–978 (1977).
19. Burakoff, S. J., Germain, R. N. & Benacerraf, B. *J. exp. Med.* **144**, 1609–1620 (1976).
20. Holden, H. T., Landolfo, S. & Herberman, R. B. *Transplant. Proc.* **9**, 1149–1152 (1977).
21. Holden, H. T. & Herberman, R. B. *Nature* **268**, 250–252 (1977).
22. Burton, R. C., Chism, S. E. & Warner, N. L. *J. Immun.* **118**, 971–980 (1977).
23. Ting, C. C. & Law, L. W. *J. Immun.* **118**, 1259–1264 (1977).

Complete protection from low-dose streptozotocin-induced diabetes in mice

STREPTOZOTOCIN (SZ) is a broad-spectrum antibiotic with oncolytic, oncogenic and diabetogenic properties^{1–4}. To produce diabetes in experimental animals, SZ is administered as a single bolus, injected either intravenously (i.v.) or intraperitoneally (i.p.). Within 72 h, selective destruction of pancreatic beta cells and hyperglycaemia occur^{3,5}. Subdiabetogenic doses of SZ cause only mild degranulation of the β -cells without hyperglycaemia during the first 72 h, unlike multiple (five) subdiabetogenic (i.v. or i.p.) doses of SZ to Charles River Laboratory (CD-1) mice, which produce marked glucose elevations within 5–6 days of the last injection of SZ, associated with morphological evidence of insulinitis^{6,7}. To dissociate direct β -cell toxicity of SZ from the delayed appearance of the insulinitis and hyperglycaemia, we used antilymphocyte serum and 3-O-methyl-D-glucose (3-OMG), a nonmetabolised glucose analogue, which prevents the occurrence of hyperglycaemia for at

least 72 h, when administered to mice or rats prior to a single diabetogenic dose of SZ^{8,9}. Antilymphocyte serum (ALS), while not altering the multiple subdiabetogenic SZ-induced hyperglycaemia, does protect against the lymphocytic infiltration of the pancreatic islets, without changing the delayed hyperglycaemic response observed following the combination of 3-OMG and SZ injections¹⁰. We report here that injections of ALS (for 5 weeks), in combination with a regimen of 3-OMG and SZ, prevented the development of hyperglycaemia for the entire 14 weeks of the experiment, and that mice demonstrated essentially normal intraperitoneal glucose tolerance tests and pancreatic histology during the 3rd and 4th months following SZ.

Four groups of mice were studied. Group 1 ($n = 4$) received five daily i.p. injections of SZ (40 mg per kg body weight). Group 2 ($n = 6$) received five daily injections of SZ and, in addition, i.p. ALS, 0.25 ml three times daily for a total of 5 weeks, including the period when SZ was administered. Group 3 ($n = 6$) received i.p. 3-OMG (1g per kg) before each of the five SZ injections. Group 4 ($n = 14$) received the combination of 3-OMG plus SZ and ALS.

Figure 1 represents the results of plasma glucose (PG) determinations. Mice receiving multiple injections of SZ alone (Group 1) showed an increase in plasma glucose (PG) by the second week. Mice injected with the combination of SZ and ALS (Group 2) had lower PG concentrations during the period of ALS administration, afterwards increasing to levels comparable to the group that received SZ alone. The group that received 3-OMG plus SZ (Group 3) showed no increase in PG for approximately 2 weeks, then PG rose to a level slightly lower than that observed in animals that received SZ alone or SZ plus ALS. In marked contrast to these three groups, the mice injected with the combination of SZ, 3-OMG and ALS (Group 4) remained normoglycaemic for the entire 14 weeks of the experiment.

Glucose tolerance tests for Group 4 were not significantly different from those of the normal controls (Fig. 2). Histological examination (9th week ($n = 5$) and the 14th week

Fig. 1 Plasma glucose levels (mean $\pm$ s.e.m.) for 14 weeks in CD-1 mice after: five daily i.p. injections of SZ (40 mg per kg) (■); ALS injections (0.25 ml) three times per week for 5 weeks plus SZ (▼); 3-OMG (1 g per kg) before each SZ dose (●); 3-OMG, plus SZ plus ALS regimen (▲). Numbers in parentheses indicate the number of mice in each group. The mice, histological examination, collection, and assaying glucose levels, preparation and administration of 3-OMG, SZ, and ALS, are as previously described¹⁰. GTT, i.p. glucose tolerance test.

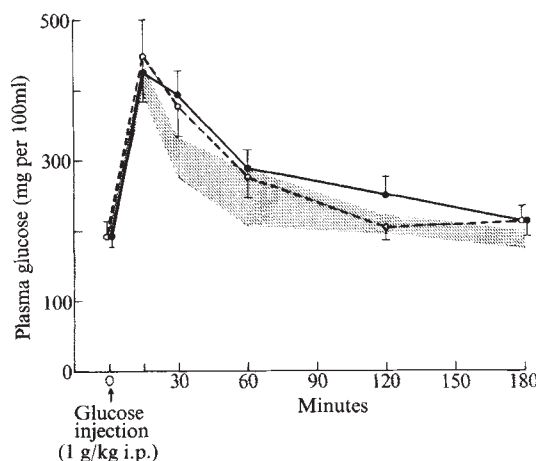
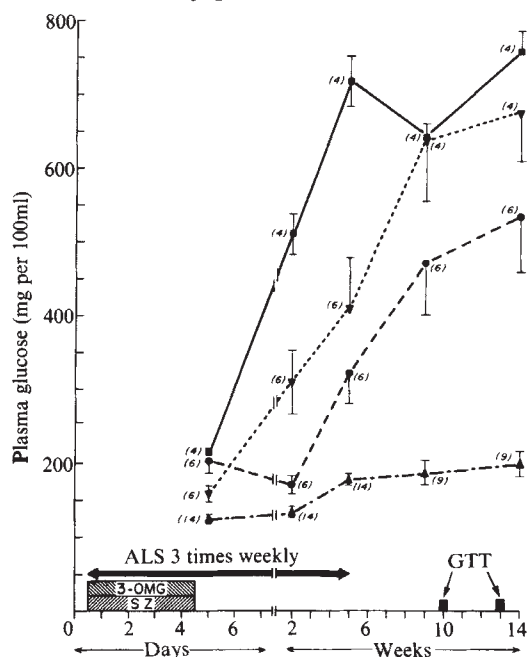


Fig. 2 I.p. glucose tolerance test. Plasma glucose response following i.p. injection of glucose (1 g per kg) in a volume of 1 ml, in animals which received the combination of 3-OMG, SZ and five weeks of ALS. The tests on experimental animals were performed during the 10th week and 13th week of the experiment. Also shown are the plasma glucose levels of five normal mice given i.p. glucose (1 g per kg) (stippled line). Numbers in parentheses indicate the number of mice in each group. ○, At 10 weeks ($n = 9$); ●, at 13 weeks ($n = 9$).

($n = 9$)) of the pancreatic islets revealed subtle architectural disarray, occasional foci of infiltrating mononuclear cells, and mild β -cell degranulation at both time intervals. Islets from Group 4 did not show the marked lymphocytic infiltration or β -cell destruction observed previously in mice receiving SZ alone^{6,7}.

A plausible explanation of the protection provided by 3-OMG against the β -cell toxicity of SZ is that of direct competition between 3-OMG and SZ for a surface receptor site on the β cell⁹. In mice, a single injection of 3-OMG (2 g per kg) prior to SZ (up to a dose of 180 mg per kg) prevents β -cell necrosis and diabetes for up to 72 h⁸. The quantity of 3-OMG used in the present experiments should provide complete protection against each of the five injections of SZ (40 mg per kg). However, as previously observed⁶, the administration of 3-OMG (1 g per kg) immediately before each of five consecutive daily doses of SZ (40 mg per kg) provided protection against glucose elevations for only approximately 2 weeks, after which the animals became diabetic, which correlated with the presence of insulinitis¹⁰. We suggest that either 3-OMG is unable to protect the β cell completely from direct SZ toxicity, or that SZ initiates a response which is only partially affected by 3-OMG pre-injection.

SZ-treated animals were given ALS to see if insulinitis and/or hyperglycaemia could be attenuated by prolonged immunosuppression. In previous experiments, ALS administered for 10 consecutive days (that is, during the five daily injections of SZ, plus 5 days thereafter) produced no appreciable diminution in plasma glucose levels when compared with animals receiving SZ alone. However, the ALS-treated mice showed no insulinitis and the combination of 3-OMG, SZ and ALS (10 injections) did not provide additional protective effect beyond that attributable to the five daily doses of 3-OMG alone¹⁰. The occurrence of diabetes in the ALS-treated animals might be considered paradoxical if our hypothesis of an immunological basis for the multidose SZ diabetes syndrome is correct unless the immunosuppression by ALS was inadequate to prevent the delayed β -cell destruction.

In the present study and in others using the inbred C57BL/KsJ mouse ($n = 10$) (data not shown), more prolonged administration of ALS (5 weeks) prevented diabetes. One explanation is that in the mice treated with ALS for only 10 days, the autoimmune state capable of destroying the β cells, was only temporarily suppressed by the ALS treatment. In contrast, the prolonged injection of ALS for five weeks suppressed the

autoimmune state to a greater extent, islet (β cell) injury was minimised and physiological impairment of glucose tolerance did not occur during the 14 weeks of the experiment.

If direct β -cell toxicity by SZ is not the only mechanism by which the animals eventually became diabetic, the protection afforded by ALS at any time would be difficult to explain. We can assume that ALS must have protected the mice against the destructive effects of the postulated cell-mediated immune reactions against the β cell. The immunosuppressive mechanism of β -cell destruction remains to be elucidated.

SZ-induced diabetes can be ameliorated by simultaneous administration of 3-OMG and prolonged administration of ALS. We suggest that 3-OMG reduces the direct β cytotoxicity of SZ, and permits the antilymphocyte effects of ALS to be expressed by inhibiting the inflammatory islet lesions and the diabetic syndrome. Our results strengthen the hypothesis that cell-mediated autoimmunity plays a role in the pathogenesis in the model of SZ-induced insulinitis.

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- Herr, R. R., Eble, T. E., Bergy, M. E. & Jahnke, H. K. *Centibiot. Ann.* **23**, 6–240 (1959–60).
- Evans, J. S., Gerittsen, G. C., Mann, K. M. & Owen, S. P. *Cancer chemother. Rep.* **48**, 1–6 (1965).
- Rakietan, N., Rakietan, M. L. & Nadkarni, M. R. *Cancer Chemother. Rep.* **29**, 91–98 (1963).
- Rakietan, N., Gordon, B. S., Beaty, A., Cooney, D. A. & Schein, P. S. *Proc. Soc. exp. Biol. Med.* **151**, 356–361 (1976).
- Rerup, C. C. *Pharmac. Rev.* **22**, 485–518 (1970).
- Like, A. A. & Rossini, A. A. *Science* **193**, 415–417 (1976).
- Like, A. A., Appel, M. C., Williams, R. M. & Rossini, A. A. *Lab. Invest.* **38**, 470–486 (1978).
- Wick, M. M., Rossini, A. A. & Glynn, D. *Cancer Res.* **37**, 3901–3903 (1977).
- Ganda, O. P., Rossini, A. A. & Like, A. A. *Diabetes* **25**, 595–603 (1976).
- Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C. & Cahill, G. F., Jr *Proc. natn. Acad. Sci. U.S.A.* **74**, 2485–2489 (1977).

Sulphonylureas increase the number of insulin receptors

SULPHONYLUREA drugs have been widely used in the management of the form of diabetes mellitus which develops in adulthood. Although some endogenous insulin secretory capacity seems to be necessary for sulphonylureas to be effective (they are ineffective in pancreatectomised animals), their precise mechanism of action has not been clear. Short-term administration of sulphonylureas to animals or humans is associated with increased insulin secretion^{1,2}. However, prolonged administration reduces pancreatic insulin content in animals^{3,4}

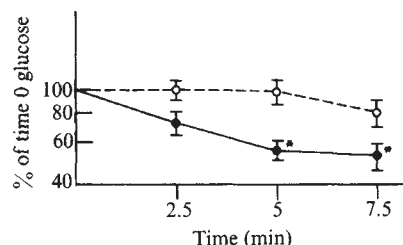


Fig. 1 Potentiation of insulin action by glipizide treatment in normal mice. 15–20-g male mice were treated with diet + glipizide (5 mg per kg per d) or diet alone for 10 d. Following a 5-h fast, the mice were injected intravenously with 2.5 mU pork insulin and killed at the specified times for measurement of plasma glucose. Each data point is the mean $\pm$ s.e. of five observations. The data are expressed as % of plasma glucose at time 0. Insulin had no effect on the plasma glucose in the control mice, but caused significant hypoglycaemia in the glipizide-treated mice at 5 and 7.5 min. $\circ$, Control; $\bullet$, glipizide; * $P < 0.05$.

and diminished insulin secretion in response to nutrient stimulation in both laboratory animals⁵ and humans with adult-onset (insulin-independent) diabetes^{6,7}. Treatment with sulphonylureas also decreases the biosynthesis of proinsulin⁸. Thus, the chronic antidiabetic action of sulphonylureas does not seem to result from an effect on insulin release, but rather from one or more extrapancreatic effects^{9,10}. In that connection, we have shown that chronic administration of the sulphonylurea, glipizide, to patients with adult-onset diabetes mellitus results in significant potentiation of insulin action, as assessed by changes in insulin-mediated glucose disposal¹¹. On the basis of those results, we have suggested that glipizide influences the interaction of insulin with the cell membranes in insulin-sensitive tissues. We now report that, in an animal model, such an effect involves an increase in the number of insulin receptors.

Male Dublin strain mice (15–20 g) were fed measured amounts of a standard diet. Glipizide (5 mg per kg per d) was added to the diet of half of them for 10 d. Weight and content of glucose and insulin in the plasma during fasting were not significantly different in controls and in mice receiving glipizide. However, sensitivity to insulin was enhanced by glipizide (Fig. 1); 2.5 mU of pork insulin did not significantly alter the plasma glucose of controls, but significant hypoglycaemia occurred in glipizide-treated mice 5 min after administration of insulin. Moreover, the concentration of insulin in the plasma at each of the times shown in Table 1 demonstrates that glipizide had no effect on insulin degradation and that insulin action was potentiated at physiological insulin concentrations. Thus, treatment of normal mice with glipizide potentiated the action of insulin on glucose disposal in a similar manner to that demonstrated in humans with diabetes mellitus.

We next investigated whether this potentiation of insulin action was accompanied by a change in the characteristics of insulin binding to the membranes of insulin-sensitive tissue. A second group of mice was given a standard diet with or without glipizide. After 10 d the livers were removed and homogenised, and purified plasma membranes were isolated by ultracentrifugation on a 15–55% linear sucrose gradient. Fractions

Table 1 Plasma insulin in mice following an intravenous dose of 2.5 mU of regular insulin

Time (min)	Glipizide treatment (μ U ml ⁻¹)	Control (μ U ml ⁻¹)
0	<2.5	<2.5
2.5	27.3 $\pm$ 4.2	30.5 $\pm$ 6.8
5	14.4 $\pm$ 2.3	11.0 $\pm$ 1.4
7.5	5.8 $\pm$ 1.0	7.0 $\pm$ 1.5

Values are mean $\pm$ s.e. of five animals.

containing plasma membranes were identified by measurement of 5' nucleotidase activity¹², and those containing endoplasmic reticulum were identified by measurement of NADH oxidase activity¹³. Protein concentrations were determined by the method of Lowry¹⁴. The specific activity of 5' nucleotidase in the pooled plasma membrane fractions was 15 times that in the crude homogenate. Enzyme profiles and specific activities were identical for the livers of all mice. Insulin binding to plasma membranes was determined by incubation of membranes for 15 h at 4°C with biologically active ¹²⁵I-insulin and various concentrations of unlabelled insulin¹⁵.

Figure 2 shows a representative experiment comparing (by Scatchard analysis¹⁶) insulin binding to purified hepatic plasma membranes from a glipizide-treated mouse with binding from a control mouse. The shapes of the two curves are similar, indicating that glipizide did not alter affinity for insulin. In contrast, the liver membranes of the treated mouse bound twice as much insulin as those of the control. ¹²⁵I-growth-hormone binding was assessed in the same way, using samples of liver membrane from the preparations already used for insulin binding. There was no difference between control and glipizide-treated mice in growth-hormone binding or displacement.

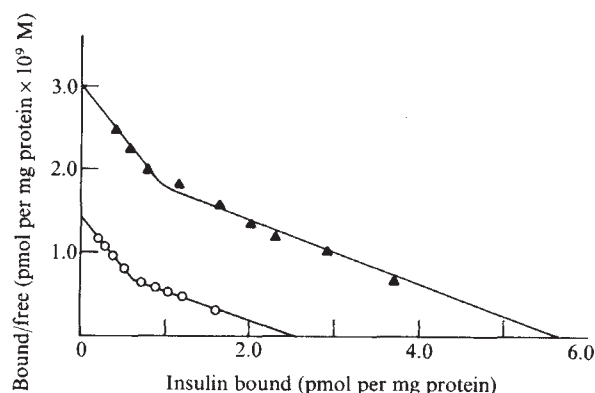


Fig. 2 Scatchard plot of insulin binding by purified hepatic plasma membranes from a glipizide-treated mouse and a control mouse. Binding studies were done by the method of Kahn *et al.*¹⁵. ○, Control; ▲, glipizide.

Table 2 summarises data calculated from four experiments similar to that depicted in Fig. 2. The Scatchard plots are curvilinear, including either negative cooperativity, binding sites of different affinities, or both. To facilitate analysis, we assumed that the first part of the curve represented a high-affinity binding site and the second part a low-affinity site. However, our conclusions do not depend on a choice between alternative mechanisms for curvilinearity. There is no significant difference in the affinity constant (K_a) between the control and glipizide-treated mice for either the high or low affinity receptors, but glipizide-treated animals have more than twice as many receptors.

Our findings provide a basis for understanding the effects of glipizide on patients with adult-onset diabetes mellitus. These patients have a marked resistance to insulin action¹⁷, which is probably a primary defect. Although the mechanism involved has not been elucidated, abnormally low numbers of insulin receptors have been found in such insulin-resistant states as adult-onset diabetes¹⁸ and obesity¹⁹. Olefsky and Reaven reported that when plasma glucose was decreased by treatment with sulphonylurea in a group of adult-onset diabetic patients, the number of insulin receptors on their circulating monocytes increased¹⁸. Our observation that successful treatment with glipizide is associated with a potentiation of insulin action on glucose disposal¹¹ is consistent with a glipizide-induced increase in the number of available insulin receptors on the plasma membranes of responsive tissues. Our data suggest that this effect is not secondary to a decrease in plasma glucose.

Table 2 Effect of glipizide treatment on purified hepatic plasma membrane insulin receptors

Treatment	High affinity receptor		Low affinity receptor	
	Receptor no. (pmol per mg)	K_a (10^9 m^{-1})	Receptor no. (pmol per mg)	K_a (10^9 m^{-1})
None	1.4 ± 0.1	1.0 ± 0.2	2.1 ± 0.1	0.4 ± 0.1
Glipizide (5 mg per kg per d)	3.2 ± 0.6*	0.9 ± 0.2	4.6 ± 1.0*	0.4 ± 0.1

Data are mean ± s.e. of four separate experiments.

* $P < 0.05$.

Our results suggest that *in vivo* glipizide treatment increases the number of insulin receptors on the target cell membrane, enhancing sensitivity to insulin²⁰. The number of plasma membrane insulin receptors *in vivo* is influenced by many factors, and our data do not enable us to delineate the precise way in which glipizide acts. Possibilities under study include direct effects on the plasma membrane, an increase in target cell cyclic AMP, which might increase the number of insulin receptors²¹, or an alteration in the mechanism by which insulin modifies the characteristics of its own receptor.

Although our results represent an important step towards understanding the mechanism of action of sulphonylurea drugs, their clinical value can be determined only by analysis of epidemiological studies comparing long-term benefits and risks. We do not know whether sulphonylureas increase insulin receptors and insulin sensitivity in all patients with adult-onset diabetes or in only a subset of patients, and the duration of the effect is unknown. Our data do, however, have practical importance in guiding the development of new drugs which influence membrane function and insulin action and in amplifying the concept of insulin resistance as a major metabolic defect in adult-onset diabetes mellitus.

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- Pfeiffer, E. F., Pfeiffer, M., Ditschuneit, H. & Ahn, C. *Ann. N.Y. Acad. Sci.* **82**, 479–495 (1959).
- Yalow, R. S., Black, H., Villazon, M. & Berson, S. A. *Diabetes* **9**, 356–362 (1960).
- Sodoyez, J. C., Sodoyez-Goffaux, F. & Foa, P. P. *Diabetes* **19**, 603–609 (1970).
- Schauder, P., Arends, J. & Frerichs, H. *Metabolism* **26**, 9–15 (1977).
- Dunbar, J. C. & Foa, P. P. *Diabetologia* **10**, 27–35 (1974).
- Reaven, G. & Gray, J. *Diabetes* **16**, 487–492 (1967).
- Duckworth, W. C., Solomon, S. S. & Kitabchi, A. E. *J. clin. Endocr. Metab.* **35**, 585–591 (1972).
- Levy, J. & Malaisse, W. J. *Biochem. Pharmacol.* **24**, 235–239 (1975).
- Feldman, J. M. & Lebovitz, H. E. *Archs intern. Med.* **123**, 314–322 (1969).
- Levey, G. S. *Fedn Proc.* **36**, 2720–2723 (1977).
- Lebovitz, H. E., Feinglos, M. N., Bucholtz, H. K. & Lebovitz, F. L. *J. clin. Endocr. Metab.* **45**, 601–604 (1977).
- Solyom, A. & Trams, E. G. *Enzyme* **13**, 329–372 (1972).
- Mackler, B. *Meth. Enzym.* **10**, 261–263 (1967).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
- Kahn, C. R., Freychet, P., Roth, J. & Neville, D. M., Jr *J. biol. Chem.* **249**, 2249–2257 (1974).
- Scatchard, G. *Ann. N.Y. Acad. Sci.* **51**, 660–672 (1949).
- Reaven, G. M., Bernstein, R., Davis, B. & Olefsky, J. M. *Am. J. Med.* **60**, 80–88 (1976).
- Olefsky, J. M. & Reaven, G. M. *Am. J. Med.* **60**, 89–95 (1976).
- Archer, J. A., Gorden, P. & Roth, J. *J. clin. Invest.* **55**, 166–174 (1975).
- Kahn, C. R. & Roth, J. in *Hormone-Receptor Interaction: Molecular Aspects* (ed. Levey, G. S.) (Marcel Dekker, New York, 1976).
- Thomopoulos, P., Kosmakos, F. C., Pastan, I. & Lovelace, E. *Biochem. biophys. Res. Commun.* **75**, 246–253 (1977).

Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine

NORADRENERGIC neurones of the locus coeruleus (LC; A6 of ref. 1) are inhibited by the systemic or local administration of adrenergic agonists²⁻⁵, opiates^{6,7} and enkephalins^{8,9}. The adrenergic receptors of the LC, which are of the presynaptic or α_2 type⁵, seem to mediate inhibitory responses to recurrent LC collaterals¹⁰ and adrenaline inputs from the lower brain stem^{4,11}. In addition to adrenergic receptors, there are opiate receptors located in the LC^{12,13} which presumably mediate the actions of endogenous opiate-like substances (enkephalins and β -endorphin) located in nerve terminals within the LC¹⁴⁻¹⁶. Recently, clonidine, which is the most powerful of the α_2 agonists known to inhibit the firing of LC neurones^{3,5}, has been reported to suppress the symptoms of opiate withdrawal in humans¹⁷. On this basis, the latter authors suggested that the opiate-withdrawal syndrome may be due in part to increased noradrenergic neuronal activity in areas such as the LC which are regulated by both α_2 adrenoceptors and opiate receptors. In this connection, it is interesting that the drug piperoxane, which blocks α_2 adrenoceptors and accelerates the firing of LC neurones^{4,5} produces many of the symptoms (such as anxiety and hypertension) seen in opiate withdrawal¹⁸. In the present study, single-cell recording and microiontophoretic techniques were used to establish the development of tolerance of LC cells to the depressant effects of morphine, naloxone-induced withdrawal activations of LC neuronal firing in morphine-dependent animals, and the ability of clonidine to suppress the withdrawal of LC neurones through a non-opiate receptor.

Thirty male albino rats (Charles River) weighing 225–275 g were used. The animals were anaesthetised with chloral hydrate (400 mg per kg, intraperitoneally) and mounted in a stereotaxic instrument. Morphine tolerance and dependence were induced by the method of subcutaneous pellet implantation^{19,20}. The pellets, which contained 75 mg of morphine base, were implanted daily for 1–5 d. For the single LC-cell recordings, a burr hole was made 1.2 mm posterior to lambda and 1.1 mm lateral to the midline. As described previously^{2-6,10}, practical aids in finding the LC include: (1) depth 5.5–6.5 mm below dural surface; (2) a zone of relative electrical silence ventral to the cerebellum and dorsal to the LC corresponding to the fourth ventricle; (3) the presence just lateral to the LC of cells of the mesencephalic nucleus of the fifth nerve, which are activated by displacement of the mandible; (4) in the LC itself, a closely packed population of slowly firing cells all responding to noxious stimulation by a burst of firing followed by a long quiescent period (~1 s). Ultimately, the recording sites were marked at the end of each experiment by iontophoretically ejected dye (Pontamine sky blue) for histological confirmation of LC and other recording sites.

Procedures for single-cell recording and microiontophoresis were as described previously⁵. The 5-barrel micropipettes which were used were broken to a tip size of 3–5 μ m; impedances of the recording (central) barrels were 3.5–5.5 M Ω (filled with 2 mM NaCl containing 2% Pontamine sky blue). Solutions for microiontophoresis were as follows: morphine sulphate, 0.1 M, pH 4 (Merck); piperoxane, 0.1 M pH 4 (Rhône-Poulenc); clonidine HCl, 0.01 M in 0.1 M NaCl, pH 4 (Boehringer-Ingelheim); naloxone HCl, 0.05 M, pH 4 (Endo). Note that, because of its extreme potency in inhibiting LC neurones, the clonidine was ionically diluted with NaCl to reduce its transport number³. A retaining current of –10 nA was applied to all drug barrels between periods of ejection. Per cent inhibition was determined by the average rate for the 1-min period immediately following iontophoretic ejection; baseline rate was taken from the 1-min period just before ejection.

The firing of LC neurones slowed gradually and became totally suppressed within 1–2 h of implantation of a single

morphine pellet ($n = 4$ animals); activity remained suppressed for about 24 h, and returned towards normal rates by 48–72 h. This pattern is consistent with the fact that morphine is released most rapidly from implanted pellets within the first 24 h^{19,20}. LC cells could be activated by a noxious stimulus during periods of morphine inhibition, as has been reported previously⁶. Twenty-four hours after the last of 4–5 daily pellet implantations, the firing of randomly encountered LC neurones (mean rate 1.2 spikes s^{-1} , $n = 20$ cells) was nearly as high as in control animals (mean rate 1.4 spikes s^{-1} , $n = 20$ cells). The difference between LC firing rates in chronic morphine-treated and control animals was not statistically significant ($P > 0.1$, Student's t test), indicating that the morphine pellet implantation procedure had induced a considerable degree of tolerance. In control animals, the microiontophoretic application of morphine at a low ejection current (+5 nA) produced marked inhibitory effects (87% mean depression, $n = 10$ cells) on LC neurones; at low ejection currents naloxone totally blocked these effects of morphine but did not increase baseline firing rates (Fig. 1a).

At a low ejection current (+5 nA) clonidine caused a similar degree of depression in LC cell firing (91% mean depression, $n = 10$ cells) despite the fact that the effect of morphine had been totally blocked by the concurrent microiontophoresis of naloxone (Fig. 1a). Conversely, the α antagonist piperoxane blocked the effect of clonidine without altering the response to morphine

Fig. 1 The effects of microiontophoretically applied morphine and clonidine on the firing of LC neurones in control and morphine-dependent rats. In *a*, taken from a recording in a control animal, the initial marked inhibitory effect of morphine (MOR) was totally blocked by naloxone; clonidine (CLON) had its usual inhibitory effect even in the presence of naloxone. In *b*, also taken from a control animal, piperoxane (PIP) selectively blocked the inhibition produced by clonidine; the response to morphine after piperoxane was virtually identical to the original response. In *c*, taken from a morphine-dependent animal (24 h after five consecutive daily pellet implantations), the initial inhibition produced by morphine was only about 50%; an activation of firing was induced by naloxone iontophoresis, during which time the inhibitory effect of morphine but not clonidine was blocked. The records represent average rate histograms generated by the analog output of an electronic counter (10 s reset time). Periods of microiontophoretic ejection are indicated by bars above the rate records; the numbers above bars give ejection currents in nA.

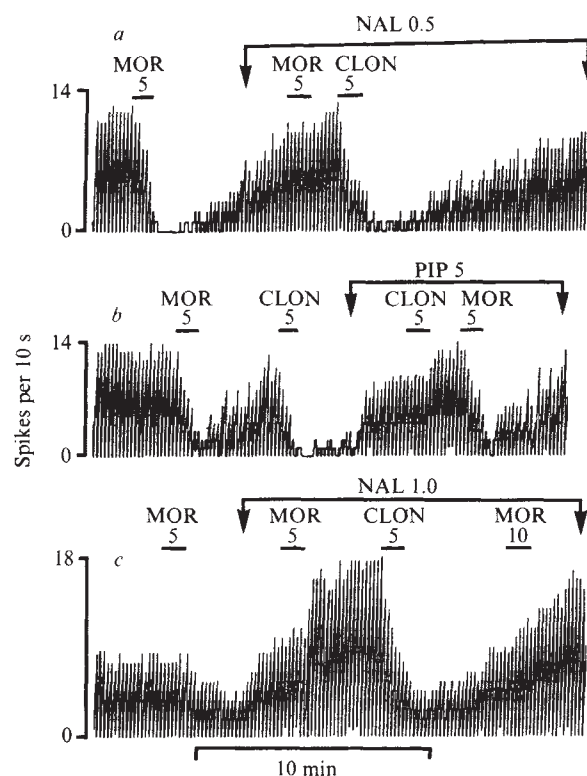


Table 1 Incidence of neuronal responses to microiontophoretically applied morphine and naloxone in LC and adjacent areas of control and morphine-dependent rats

Preparation	Region (no. of cells)	Morphine		Naloxone	
		% Cells activated	% Cells depressed	% Cells activated	% Cells depressed
Control rats (<i>n</i> = 5)	Locus coeruleus (<i>n</i> = 10)	0	100	0	0
	Cerebellum, Purkinje cells (<i>n</i> = 6)	33	0	0	0
	Mesencephalic nucleus of fifth nerve (<i>n</i> = 5)	0	0	0	0
	Reticular formation/central grey (<i>n</i> = 10)	10	10	0	0
Morphine-dependent rats (<i>n</i> = 5)	Locus coeruleus (<i>n</i> = 10)	0	100	100	0
	Cerebellum, Purkinje cells (<i>n</i> = 5)	40	0	0	0
	Mesencephalic nucleus of fifth nerve (<i>n</i> = 5)	0	0	0	0
	Reticular formation/central grey (<i>n</i> = 12)	8	8	8	8

In all cases, microiontophoretic currents were: +5 nA, morphine; +0.5–1.0 nA, naloxone. Morphine was applied for 1 min; naloxone was applied 3 or more min. Purkinje cells were identified by climbing fibre bursts. The mesencephalic nucleus of the fifth nerve was identified by activation caused by displacement of mandible.

(*n* = 5 cells; Fig. 1*b*). Figure 1*c* shows that in morphine-dependent rats (24 h after 4–5 daily pellet implantations) the inhibitory response of LC cells (*n* = 10) to microiontophoretic morphine was reduced compared with the controls (54% compared with 87%; *P* < 0.01, Student's *t* test). In all the dependent animals, the sustained microiontophoresis of naloxone on to LC cells produced a dramatic increase over baseline firing rate (mean 126%, *n* = 10 cells; Fig. 1*c*). Following naloxone, responses to microiontophoretically applied morphine were abolished. However, even during periods of naloxone-induced opiate-receptor blockade and withdrawal activation, the microiontophoresis of clonidine was still able to depress LC cell firing to below baseline rates (Fig. 1*c*). The uniform responses of LC cells to morphine and naloxone in both control and dependent animals were in sharp contrast to the relatively low incidence of effects in cells in areas immediately surrounding the LC (Table 1).

The first part of this study shows that tolerance develops to the powerful inhibitory effect of morphine on the firing of LC neurones. In fact, 24 h after 4–5 daily morphine pellet implantations, LC cells fired in a normal range, whereas 24 h after a single pellet spontaneous activity was markedly suppressed. In tolerant animals the direct application of naloxone to LC neurones by microiontophoresis induced a withdrawal response characterised by a >100% activation of firing. In other experiments (not reported above) the intravenous administration of naloxone (0.02 mg per kg) to dependent (but not control) rats also produced a dramatic increase in LC cell firing; this increase was reversed by intravenous clonidine. However, because of the generalised nature of the withdrawal reaction produced by systemically administered naloxone, it cannot be known with certainty that the effects of LC cell firing represent a direct action within the LC. It has been reported that the iontophoretic application of naloxone to cortical neurones in morphine-dependent rats induces an increase in neuronal firing²¹. In the present study, at the same low microiontophoretic ejection currents which were used in the LC, most neurones in areas immediately surrounding the LC (cerebellum, mesencephalic nucleus of the fifth nerve, central grey/reticular formation) were not responsive to morphine and did not display naloxone-induced withdrawal. The marked sensitivity and high selectivity of LC neurones for opiates and opiate antagonists in this region of the brain is consistent with previous studies^{6–8}.

The results of this study also indicate that morphine and clonidine act at independent LC-cell receptors, as the opiate antagonist naloxone blocks morphine but not clonidine whereas

the α antagonist piperoxane blocks clonidine but not morphine. Previous studies, in which morphine and D-amphetamine were administered by the systemic route, also suggest that opiate receptors and adrenergic receptors which regulate LC cells are distinct, as naloxone blocked the LC-inhibitory action of morphine but not that of the indirectly acting adrenergic agent D-amphetamine⁶. The present study extends this work by showing that specifically within the LC itself naloxone antagonises morphine but not the α_2 -adrenergic agonist clonidine. In addition, reciprocal selectivity has been shown by the fact that the α blocker piperoxane antagonised clonidine but not morphine. Although morphine and clonidine thus seem to act on independent receptors within the LC, it is notable that they have similar depressant effects on overall LC cell activity. These observations provide a basis for the proposal that the α_2 agonist clonidine might suppress certain symptoms of opiate withdrawal by means of a parallel but independent action on cell activity¹⁷. Moreover, a perpetuation of a state of dependence is avoided as clonidine acts on a non-opiate receptor. Of course there are opiate receptors at diverse sites within the central nervous system^{12,13,22}, some of which presumably do not overlap with the location of α_2 adrenoceptors. Nevertheless, the effectiveness of clonidine in suppressing opiate withdrawal in humans suggests that significant components of the opiate-withdrawal syndrome may be due to increased noradrenergic activity in areas such as the LC which are affected in a like manner by both α_2 adrenoceptors and opiate receptors¹⁷. It is also possible that at some sites clonidine could suppress opiate withdrawal through an action on non-adrenergic receptors (for example, histamine receptors²³). However, the actions of clonidine in the LC would seem to be through an α adrenoceptor as they can be blocked selectively by the α antagonist piperoxane.

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1. Dahlström, A. & Fuxe, K. *Acta physiol. scand.* **62**, Suppl. 232, 1–55 (1965).
2. Graham, A. W. & Aghajanian, G. K. *Nature* **234**, 100–102 (1971).
3. Svensson, T., Bunney, B. S. & Aghajanian, G. K. *Brain Res.* **92**, 291–306 (1975).

4. Cedarbaum, J. M. & Aghajanian, G. K. *Brain Res.* **112**, 413–419 (1976).
5. Cedarbaum, J. M. & Aghajanian, G. K. *Eur. J. Pharmac.* **44**, 375–385 (1977).
6. Korf, J., Bunney, B. S. & Aghajanian, G. K. *Eur. J. Pharmac.* **25**, 165–169 (1974).
7. Bird, S. J. & Kuhar, M. J. *Brain Res.* **122**, 523–533 (1977).
8. Young, W. S., Bird, S. J. & Kuhar, M. J. *Brain Res.* **129**, 366–370 (1977).
9. Guyenet, P. G. & Aghajanian, G. K. *Brain Res.* **136**, 178–184 (1977).
10. Aghajanian, G. K., Cedarbaum, J. M. & Wang, R. Y. *Brain Res.* **136**, 570–577 (1977).
11. Fuxe, K., Lidbrink, P., Hökfelt, T., Bolme, P. & Goldstein, M. *Acta physiol. scand.* **91**, 566–567 (1974).
12. Pert, C. B., Kuhar, M. J. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3729–3733 (1976).
13. Atweh, S. F. & Kuhar, M. J. *Brain Res.* **129**, 1–12 (1977).
14. Simantov, R., Kuhar, M. J., Uhl, G. R. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2167–2171 (1977).
15. Watson, S. J., Akil, H., Sullivan, S. & Barchas, J. D. *Life Sci.* **21**, 733–738 (1977).
16. Bloom, F. E. *et al. Neurosci. Abstr.* **3**, 286 (1977).
17. Gold, M. E., Redmond, D. E., Jr. & Kleber, H. D. *Lancet* **ii**, 599–602 (1978).
18. Redmond, D. E. *Jr in Animal Models in Psychiatry and Neurology* (eds Hanin, I. & Usdin, E.) 293 (Oxford, 1977).
19. Way, E. L., Loh, H. M. & Shen, F. J. *Pharmac. exp. Ther.* **167**, 1–8 (1969).
20. Bläsig, J., Herz, A., Reinhold, K. & Zieglgänsberger, S. *Psychopharmacologia* **33**, 19–38 (1973).
21. Satoh, M., Zieglgänsberger, W. & Herz, A. *Brain Res.* **115**, 99–110 (1976).
22. Atweh, S. F. & Kuhar, M. J. *Brain Res.* **124**, 53–67; **134**, 393–405 (1977).
23. Karpunen, H. O., Paakkari, I., Paakkari, P., Huotari, R. & Orma, A. L. *Nature* **259**, 587–588 (1976).

Insect acetylcholine receptors as a site of insecticide action

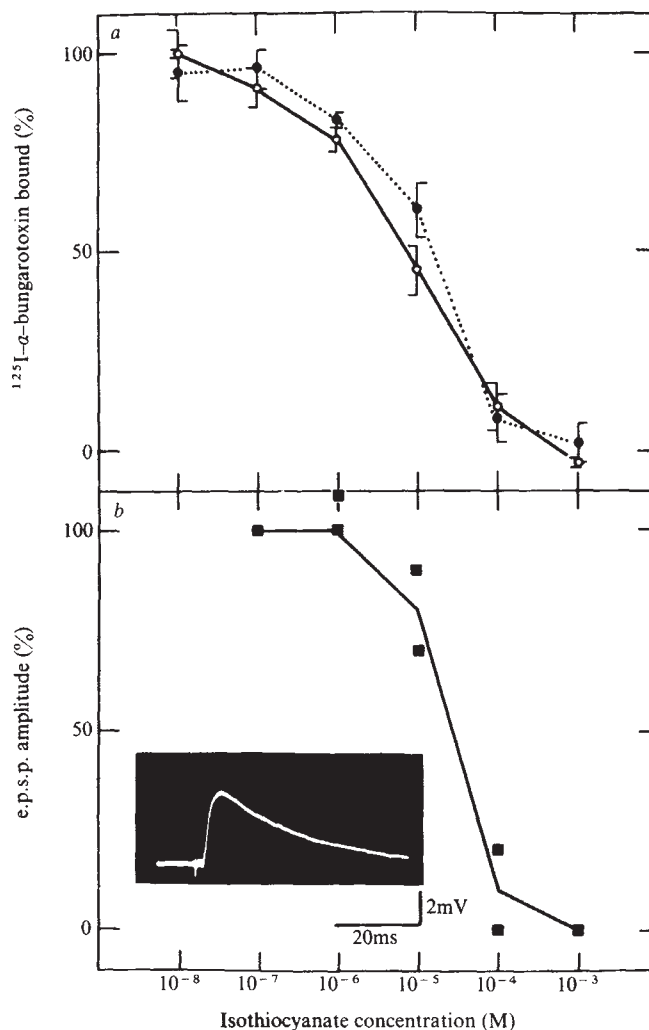
ACETYLCHOLINE is thought to be an excitatory neurotransmitter at synapses in the insect central nervous system (CNS) and does not seem to be involved in insect neuromuscular transmission^{1,2}. Most of the current generation of insecticides are inhibitors of the enzyme acetylcholinesterase which hydrolyses acetylcholine, terminating its synaptic actions³. Far less attention has been given to other components of cholinergic synapses which might constitute potential sites of action of insecticidally active molecules. These include the presynaptic synthesis of acetylcholine involving the enzyme choline acetyltransferase, the mechanism of transmitter release and the post-synaptically located acetylcholine receptor molecules. The present study demonstrates that an isothiocyanate compound and nicotine, both of which show insecticidal activity, are agonists for an acetylcholine receptor in the CNS of the cockroach *Periplaneta americana*.

2-Isothiocyanatoethyltrimethylammonium iodide, synthesised as a potential insecticide, was found to be a potent inhibitor of choline acetyltransferase partially purified from fly heads⁴. In the same study the dissociation constant for the enzyme-inhibitor complex was found to be 0.06 μ M. No significant inhibition of fly-head acetylcholinesterase was detected at a concentration of 100 μ M (ref. 4). The possibility of a cholinergic presynaptic site of action in the insect CNS was therefore tested by perfusing the terminal abdominal ganglion of *Periplaneta* with saline containing 2-isothiocyanatoethyltrimethylammonium iodide (normal saline composition in mM: NaCl, 208.6; KCl, 3.1; CaCl₂, 5.4; NaHCO₃, 2.0; pH 7.0). Transmission at the cercal-nerve giant-fibre synapses, for which acetylcholine is the putative transmitter^{5,6}, was monitored by oil-gap (single-fibre) and sucrose-gap recording techniques⁷. A slow progressive block of the evoked excitatory postsynaptic potential (e.p.s.p.) might have been expected because of the time required both for access of the compound to the intracellular enzyme choline acetyltransferase and for the utilisation of existing stores of acetylcholine. Instead, however, a rapid reduction of the evoked e.p.s.p. and postsynaptic membrane resistance was detected, accompanied by depolarisation of the postsynaptic membrane at concentrations above 10⁻⁵ M (ref. 7). These results suggested a direct action of the isothiocyanate on the postsynaptic membrane, possibly on the acetylcholine receptors. The present study was designed to test this hypothesis by examining the binding of the isothiocyanate to an insect acetylcholine receptor and comparing this quantitatively with its ability to produce postsynaptic blockade. For comparison, parallel experiments

were carried out using nicotine, which is both an insecticide and a potent ganglionic blocking agent in insects⁵.

As a receptor probe we have used ¹²⁵I- α -bungarotoxin, which binds specifically to nicotinic acetylcholine receptors of vertebrates^{8,9}. An ¹²⁵I- α -bungarotoxin-binding component with the expected properties of an acetylcholine receptor¹⁰ has been characterised in fly heads of *Drosophila melanogaster*^{11–14} and *Musca domestica*¹³, in brain tissue of the moth *Manduca sexta*¹⁵, and in abdominal nerve cords of *P. americana*¹⁶. In view of the controversy over the relationship between α -bungarotoxin-binding activity and the nicotinic acetylcholine receptor in the vertebrate CNS^{17,18}, it is important to note that α -bungarotoxin blocks transmission at the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *Periplaneta*¹⁹. To determine whether the isothiocyanate and nicotine bind to acetylcholine receptors, we have compared their ability to

Fig. 1 *a*, Concentration dependence of inhibition by 2-isothiocyanatoethyltrimethylammonium iodide of ¹²⁵I- α -bungarotoxin binding to extracts of both whole flies (*D. melanogaster*) and cockroach abdominal nerve cords (*P. americana*). Results are expressed as a percentage of toxin bound to extract in the absence of the isothiocyanate. Each point is the average of three replicates and vertical bars denote 1 s.d. Concentrations for 50% inhibition of toxin binding are estimated to be 6.9 $\times 10^{-6}$ M (*Drosophila*; $\circ$) and 1.6 $\times 10^{-5}$ M (*Periplaneta*; $\bullet$). *b*, Effects of various isothiocyanate concentrations on the amplitude of the evoked e.p.s.p. recorded from the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *P. americana* using a modified sucrose-gap technique⁷. Concentration for 50% suppression of the e.p.s.p. is estimated to be 2.6 $\times 10^{-5}$ M. Inset shows the e.p.s.p. recorded in normal saline (100%).



inhibit ^{125}I - α -bungarotoxin binding to particulate extracts with their competence to modify transmission at a synapse that can be blocked by α -bungarotoxin. Differential centrifugation techniques described previously have been used to prepare the

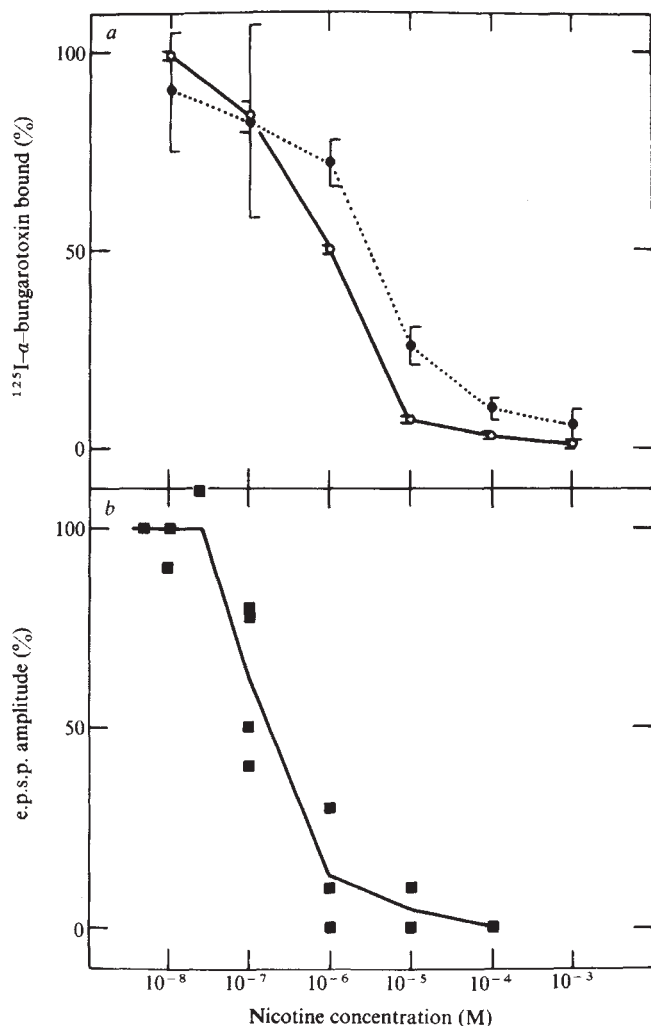


Fig. 2 *a*, Concentration dependence of inhibition by nicotine of ^{125}I - α -bungarotoxin binding to extracts of both whole flies (*D. melanogaster*) and cockroach abdominal nerve cords (*P. americana*). Results are expressed as percentage of toxin bound to extract in the absence of nicotine. Concentrations for 50% inhibition of toxin binding are estimated to be 1.0×10^{-6} M (*Drosophila*; $\circ$) and 3.0×10^{-6} M (*Periplaneta*; $\bullet$). Each point is the average of three replicates and vertical bars denote 1 s.d. *b*, Effects of various concentrations of nicotine on the amplitude of the evoked e.p.s.p. recorded from the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *P. americana*. Concentration for 50% suppression of the e.p.s.p. is estimated to be 2.0×10^{-7} M.

particulate form of the receptor from *D. melanogaster*¹¹ (20,000g pellet from whole flies) and *P. americana*¹⁶ (40,000g pellet from abdominal nerve cords). Extract equivalent to either 0.005g flies (*Drosophila*) or to 0.001g abdominal nerve cords (*Periplaneta*) was incubated with a solution of either 2-isothiocyanatoethyltrimethylammonium iodide or nicotine in Krebs original Ringer buffer, pH 7.4 (ref. 20) at 21°C for 30 min. Such mixtures were then combined with ^{125}I - α -bungarotoxin in Krebs Ringer buffer, pH 7.4, containing 0.1% bovine serum albumin and incubated in microfuge tubes (Beckman, no. 314326) at 21°C for a further 30 min, before centrifugation at 30,000g (30 min, 4°C).

After removal of the supernatant fluid, pellets were rinsed twice and counted at 80% efficiency in a gamma counter (Searle model 1197). Background binding determined as the amount of ^{125}I - α -bungarotoxin bound to heat-inactivated extract was routinely subtracted. Similar levels of background binding were noted in the presence of 10^{-3} M nicotine. 100% binding was defined as the amount of ^{125}I - α -bungarotoxin bound in the absence of added ligand. In this way it was shown that 2-isothiocyanatoethyltrimethylammonium iodide inhibited toxin binding to the insect extracts (Fig. 1*a*). The concentrations of isothiocyanate producing 50% inhibition of toxin binding (ID_{50}) were estimated to be 1.6×10^{-5} M (*Periplaneta*) and 6.9×10^{-6} M (*Drosophila*). It was also demonstrated that nicotine inhibited toxin binding somewhat more effectively than the isothiocyanate (Fig. 2*a*). The ID_{50} concentrations for nicotine were estimated to be 3.0×10^{-6} M (*Periplaneta*) and 1.0×10^{-6} M (*Drosophila*). This value obtained for *Drosophila* extract agrees with the ID_{50} value of 0.5×10^{-6} M previously estimated for the inhibition by nicotine of toxin binding to *Drosophila* extract¹¹. The ability of these insecticidally active compounds to inhibit α -bungarotoxin binding is similar to that found for acetylcholine in the presence of an anticholinesterase ($\text{ID}_{50} = 2.8\text{--}7.9 \times 10^{-6}$ M for *Drosophila*)¹¹.

In parallel electrophysiological experiments on the terminal abdominal ganglion of *Periplaneta*, changes were followed in the amplitude of the monosynaptic e.p.s.p. evoked by electrical stimulation of cercal nerves and recorded by the sucrose-gap technique from the cercal-nerve giant-fibre synapses during the perfusion of the ganglion by the test compounds. The ganglionic nerve sheath was routinely split to facilitate access to the synapses of the applied compounds⁷. It was estimated that 50% inhibition of the e.p.s.p. was produced by a concentration of 2.6×10^{-5} M isothiocyanate (Fig. 1*b*). As shown in Fig. 2*b*, the concentration of nicotine estimated to produce 50% block of the e.p.s.p. was 2.0×10^{-7} M. Just as nicotine was more effective than the isothiocyanate in inhibiting toxin binding *in vitro*, it was also a more potent inhibitor of the e.p.s.p.

We have demonstrated that 2-isothiocyanatoethyltrimethylammonium iodide produces half-maximal block of ^{125}I - α -bungarotoxin binding to the insect extracts (*Periplaneta* CNS and *Drosophila* whole flies) at concentrations similar to those producing half-maximal suppression of e.p.s.p. amplitude at a central synapse in *Periplaneta* which can be blocked by α -bungarotoxin¹⁹. Differences between the factors affecting biochemical and physiological techniques make direct comparison of absolute ID_{50} values difficult. However, within each technique the order of effectiveness of test compounds can be determined and then compared between techniques. Studies with nicotine have shown that this cholinergic ligand is more potent than the isothiocyanate both in its ability to inhibit ^{125}I - α -bungarotoxin binding and in its actions on the post-synaptic membrane. The data indicate that the synaptic actions of both these insecticidally active molecules can be accounted for by their binding to the α -bungarotoxin-sensitive acetylcholine receptors, as they both inhibit toxin binding at physiologically relevant concentrations.

Our results provide direct evidence for an insect acetylcholine receptor as a site of insecticide action and thereby emphasise the need for detailed comparative studies on the properties of insect and vertebrate cholinergic receptors. We have demonstrated that the α -bungarotoxin binding assay could provide a rapid way to screen potential insecticides for action on a cholinergic receptor. We have also, indirectly, illustrated the difficulties in attempting a rational design of insecticide molecules. It should be recalled that 2-isothiocyanatoethyltrimethylammonium iodide, which has been shown to be effective at the postsynaptically located α -bungarotoxin-sensitive acetylcholine receptor, was originally designed as an inhibitor of the presynaptically located enzyme choline acetyltransferase.

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- Gerschenfeld, H. M. *Physiol. Rev.* **53**, 1-119 (1973).
- Pichon, Y. in *The Physiology of Insecta* Vol. 4 (ed. Rockstein, M.) 101-174 (Academic Press, New York, 1974).
- Corbett, J. R. *The Biochemical Mode of Action of Pesticides* (Academic Press, London, 1974).
- Baillie, A. C., Corbett, J. R., Dowsett, J. R., Sattelle, D. B. & Callec, J.-J. *Pestic. Sci.* **6**, 645-653 (1975).
- Shankland, D. L., Rose, J. A. & Donniger, C. J. *Neurobiol.* **2**, 247-262 (1971).
- Sattelle, D. B., McClay, A. S., Dowson, R. J. & Callec, J.-J. *J. exp. Biol.* **64**, 13-23 (1976).
- Sattelle, D. B. & Callec, J.-J. *Pestic. Sci.* **8**, 735-746 (1977).
- Berg, D. K., Kelly, R. B., Sargent, P. B., Williamson, P. & Hall, Z. W. *Proc. natn. Acad. Sci. U.S.A.* **69**, 147-151 (1972).
- Potter, L. T. in *Meth. Enzym.* **32**, 309-323 (1974).
- Birdsall, N. J. M. & Hulme, E. C. *J. Neurochem.* **27**, 7-16 (1976).
- Schmidt-Nielsen, B. K., Gepner, J. I., Teng, N. N. H. & Hall, L. M. *J. Neurochem.* **29**, 1013-1029 (1977).
- Dudai, Y. & Amsterdam, A. *Brain Res.* **130**, 551-555 (1977).
- Dudai, Y. *FEBS Lett.* **76**, 211-213 (1977).
- Rudloff, E. *Expl Cell Res.* **111**, 185-190 (1978).
- Sanes, J. R., Prescott, D. J. & Hildebrand, J. G. *Brain Res.* **119**, 389-402 (1977).
- Sattelle, D. B., Gepner, J. I. & Hall, L. M. (submitted).
- Carbonetto, S. T., Fambrough, D. M. & Muller, K. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1016-1020 (1978).
- Chiappinelli, V. A. & Zigmond, R. E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2999-3003 (1978).
- Sattelle, D. B., Hue, B., Harrow, I. D., Gepner, J. I. & Hall, L. M. (submitted).
- Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. in *Data for Biochemical Research*, 2nd edn, 507 (Oxford University Press, London, 1969).

Morphine and β -endorphin influence the secretion of the endocrine pancreas

OPIOID PEPTIDES¹⁻³ are present in high concentrations in those areas of the brain and gastrointestinal tract⁴ in which opiate receptors⁵ are numerous and a biological response to opiates well established⁶. The report of immunoreactive enkephalin in the pancreas⁴ raised the possibility that a similar association between the presence of endogenous peptide and opiate receptors might exist in this organ and would be manifested by a biological response to these agents. We report here that an exogenous opiate and an endogenous opioid peptide both exert biological effects upon the endocrine pancreas that are compatible with the presence of opiate receptors in the islets of Langerhans.

We examined the effects of morphine and the opioid peptide β -endorphin on the release of immunoreactive insulin, glucagon and somatostatin from the isolated perfused dog pancreas. We used the preparation of Iversen and Miles⁷, in which the pancreas is perfused without recirculation at a rate of 20 ml min⁻¹. Somatostatin-like immunoreactivity (SLI) was measured by a radioimmunoassay using antiserum R101 of Arimura^{8,9}, and insulin and glucagon by a modification of previously described methods^{10,11}.

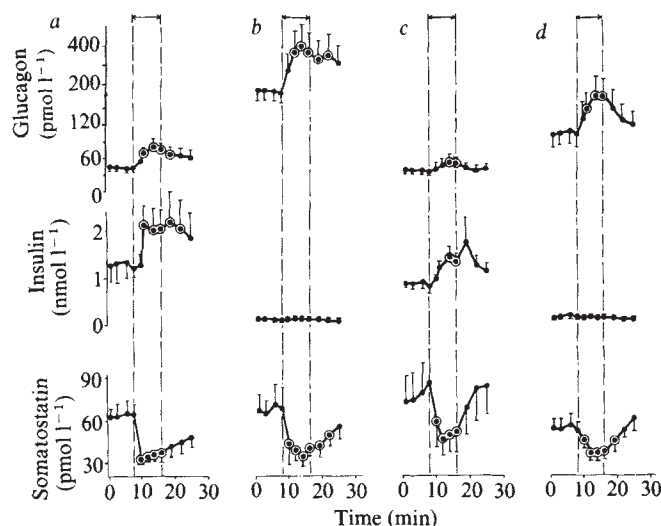
Perfusion of morphine sulphate (Wyeth) at a concentration of 3 μ mol l⁻¹ in the presence of 5.5 mM glucose lowered SLI levels by 50% ($P < 0.05$) at 2 min, while insulin and glucagon levels almost doubled ($P < 0.05$), each reaching a peak at 4-6 min (Fig. 1a). When glucose was omitted from the perfusate (Fig. 1b), SLI levels again declined by approximately 50% ($P < 0.05$); however, as expected, baseline glucagon levels were higher and

the peak glucagon level was fourfold greater than in the presence of 5.5 mM glucose, while baseline insulin concentrations were lower and did not rise measurably in response to the morphine.

We then tested the effects on islet hormone secretion of the opioid peptide, β -endorphin (synthesised at the Salk Institute by N. Ling), at a concentration of 54 nmol l⁻¹, 1/50 of the molar concentration of morphine, both in the presence of 5.5 mM glucose and in its absence. As in the case of morphine, in the presence of 5.5 mM glucose SLI release was suppressed by 50% and again statistically significant inhibition occurred within 2 min ($P < 0.05$), preceding the peak changes in insulin and glucagon (Fig. 1c). Mean glucagon concentrations rose slightly but significantly ($P < 0.05$), and insulin levels increased almost twofold, with peaks once again occurring at 4-6 min ($P < 0.05$). Omission of glucose from the perfusate was again associated with higher baseline levels of glucagon and an exaggerated glucagon response to β -endorphin ($P < 0.05$), while baseline insulin levels were lowered and the insulin response to the opioid peptide abolished. However, SLI levels were suppressed ($P < 0.05$) as in the presence of glucose (Fig. 1d).

The similarity of the islet responses to two dissimilar substances, an opiate alkaloid and an endogenous peptide, suggested that they were mediated by a specific receptor mechanism. If so, these effects should be prevented by a specific antagonist. We therefore examined the effects of the opiate antagonist, naloxone hydrochloride (Endo), perfused at 11 μ mol l⁻¹. Naloxone perfused alone did not affect either glucagon or SLI release, although insulin concentration increased significantly ($P < 0.05$) (ref. 9, Fig. 2a). Naloxone blocked the morphine-induced changes in glucagon and SLI, but the insulin response was reduced by only about 50% (Fig. 2b, left panel). The effects of β -endorphin on glucagon, somatostatin and insulin were completely abolished by the opiate antagonist ($P < 0.05$) (Fig. 2b, right panel).

Fig. 1 The effect of morphine (a, b) and β -endorphin (c, d) on immunoreactive glucagon, insulin and somatostatin release from the isolated dog pancreas. Note the change in scale on the glucagon axis. Significant differences ($P < 0.05$, ref. 9) from baseline are indicated by encircled dots. The pancreas was surgically isolated from healthy conditioned male dogs weighing 20-30 kg and then perfused with a semisynthetic buffer, as previously described⁵. The perfusate consisted of a Krebs-Ringer-bicarbonate buffer with 4% Dextran T-70 (Pharmacia), 10 mM arginine, with or without 5.5 mM glucose. Morphine sulphate or porcine β -endorphin were infused for 8-min periods on one or two occasions in each pancreas. The pancreatic effluent was collected at 1-min intervals and frozen for subsequent radioimmunoassay. a, 10 mM arginine + 5.5 mM glucose; morphine 3 μ mol l⁻¹; n = 6. b, 10 mM arginine, no glucose; morphine 3 μ mol l⁻¹; n = 6. c, 10 mM arginine + 5.5 mM glucose; β -endorphin 54 nmol l⁻¹; n = 7. d, 10 mM arginine, no glucose; β -endorphin 54 nmol l⁻¹; n = 7.



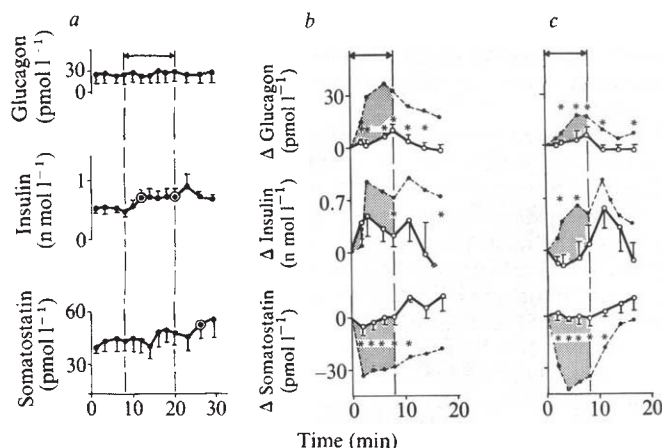


Fig. 2 *a*, The effect of naloxone alone on immunoreactive glucagon, insulin and somatostatin release from the isolated dog pancreas. Significant differences ($P < 0.05$, ref. 12) from baseline are indicated by encircled dots. *b*, The effect of naloxone on morphine and β -endorphin induced changes in islet cell function. Morphine ($3 \mu\text{mol l}^{-1}$) or β -endorphin (54 nmol l^{-1}) were perfused for 8 min, beginning 4 min after the start of the naloxone perfusion. The responses during naloxone perfusion are compared to those in the absence of naloxone, and the differences are indicated by the stippled areas. The hormone concentration immediately before the perfusion of morphine or β -endorphin was used as the baseline in order to derive the changes induced by each substance over and above changes induced by naloxone alone. Statistically significant ($P < 0.05$, ref. 13) differences between the two groups are indicated by an asterisk. The perfusate of all experiments illustrated in Fig. 2 contained 5.5 mM glucose, and each experiment was carried out in a different pancreas. *a*, 10 mM arginine + 5.5 mM glucose; naloxone $11 \mu\text{mol l}^{-1}$. *b*, Morphine; $\circ$, naloxone added; $\bullet$, no naloxone. *c*, β -Endorphin; $\circ$, naloxone added; $\bullet$, no naloxone. Values are means from six experiments.

Thus, it seems that the opiate alkaloids and endogenous opioid peptides are capable of altering islet cell function, probably via a specific receptor mechanism. A similar observation has recently been made with respect to the exocrine pancreas¹⁴, long known to be responsive to morphine¹⁵. The presence of endocrine cells with enkephalin-like immunofluorescence in islets of the pancreas¹⁶ raises the possibility that locally secreted enkephalin may influence islet cell function through nearby receptors, and that circulating β -endorphin, which is released from the corticotrophs of the pituitary in response to stress¹⁵, might also exert such effects. The failure of naloxone to produce any effect on basal SLI and glucagon release argues against a persisting 'tonic' *in vitro* action of endogenous opioid peptides that can be blocked by the antagonist, but does not exclude such activity *in vivo* in an intact pancreas.

Hyperglycaemia has been observed after morphine injection in various species^{18–20} and was attributed to central effects of the drug²¹, mediated by the adrenergic nervous system²², but no studies of the acute effects of morphine on islet hormone release have ever been reported. The possibility that direct stimulation of glucagon release may contribute to hyperglycaemia, especially in states of insulin deficiency such as diabetes mellitus, should be considered.

Finally, it should be noted that in these studies significant suppression of SLI always preceded significant changes in insulin and glucagon release. This raises the possibility that a primary event in opiate action upon the islets is suppression of endogenous somatostatin and release of insulin and glucagon from its inhibitory influence.

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- Hughes, J. *et al. Nature* **258**, 577 (1975).
- Ling, N., Burgus, R. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3942 (1976).
- Cox, B. M., Goldstein, A. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1821 (1976).
- Polak, J. M. *et al. Lancet* **i**, 972 (1977).
- Pert, C. B. & Snyder, S. H. *Science* **179**, 1011 (1973).
- Snyder, S. H. *N. Engl. J. Med.* **296**, 266 (1977).
- Iversen, J. & Miles, D. W. *Diabetes* **20**, 1 (1971).
- Arimura, A., Sata, H., Coy, D. H. & Schally, A. V. *Proc. Soc. exp. Biol. Med.* **148**, 784 (1975).
- Harris, V., *et al. Clin. chim. Acta* **87**, 275 (1978).
- Yalow, R. S. & Berson, S. A. *J. clin. Invest.* **39**, 1157 (1960).
- Faloon, G. R. & Unger, R. H. in *Methods of Hormone Radioimmunoassay* (eds Jaffe, B. M. & Behrman, H. R.) 317 (Academic, New York, 1974).
- Wilcoxon, F. *Biom. Bull.* **1**, 80–83 (1945).
- Mann, H. B. & Whitney, D. R. *Ann. math. Statist.* **18**, 50–60 (1947).
- Konturek, S. J. *et al. Gastroenterology* **74**, 851 (1978).
- Bowman, W. C., Rand, M. J. & West, G. B. in *Textbook of Pharmacology*, 635 (Blackwell, Oxford, 1968).
- Forsmann, W. G., Helmstaedter, V. & Feurle, G. *Acta hepatogastroenterologica* **24**, 488 (1977).
- Guillemin, R. *et al. Science* **197**, 1367 (1977).
- Araki, T. *Hoppe Seyler's Z. physiol. Chem.* **15**, 546 (1891).
- Ross, E. L. *J. biol. Chem.* **34**, 335 (1918).
- Jaffe, J. H. & Martin, W. R. in *The Pharmacological Basis of Therapeutics* (eds Goodman, L. S. & Gilman, A.) 250 (1975).
- Feldberg, W. & Shaligram, S. V. *Br. J. Pharmac.* **46**, 602 (1972).
- Bodo, R. C., Cotui, F. W. & Benaglia, A. E. *J. Pharmac., exp. Ther.* **61**, 48 (1927).

Induction of ornithine decarboxylase by nerve growth factor dissociated from effects on survival and neurite outgrowth

SEVERAL eukaryotic cell types respond to a variety of hormones or growth-promoting agents by a rapid induction of ornithine decarboxylase (ODC) activity (for reviews see refs 1, 2). This enzyme² and the polyamines whose synthesis it catalyses¹ have been suggested in turn to play a part in mediating subsequent cellular responses to such hormone or replication factors. Among the agents shown to affect ODC activity is nerve growth factor (NGF), a protein which affects the differentiation and survival of sympathetic and responsive sensory neurones³. MacDonnell *et al.*⁴ have demonstrated that treatment of rat superior cervical ganglia with NGF causes a rapid (maximal by 6–7 h), transcription-dependent increase in ODC activity. NGF treatment has also been reported to increase ODC activity in rat brain⁵. Such findings have led to the suggestion that ODC may mediate other responses of neurones to NGF⁴. We show here, however, that suppression of ODC activity and of its induction do not interfere with several other responses to NGF such as promotion of neurite outgrowth, maintenance of survival and increase in cell size. Such findings may be relevant to the possible role of ODC induction in other differentiating systems.

One particularly useful model system for studying the mechanism of action of NGF is a clonal line (designated PC12) of rat adrenal pheochromocytoma cells⁶. In serum-containing medium and in the absence of NGF, such cells display several differentiated properties associated with adrenal chromaffin cells^{6,7}. When treated with nanogram levels of NGF, however, PC12 cells cease replication and acquire certain features of sympathetic neurones, including the formation of extensive neurites⁶ (Fig. 3) and electrical excitability⁸. One of the advantages of the PC12 line is that it may be used to study the initial

events whereby NGF affects target cells (normal responsive neurones, in contrast, have already been exposed to the factor *in vivo* before experimental intervention). Moreover, PC12 cultures maintained with serum can be used as adequate non-NGF-treated controls (normal sympathetic neurones, in contrast, require NGF for survival⁹ and hence, NGF-untreated material may be dying and unsuitable as controls).

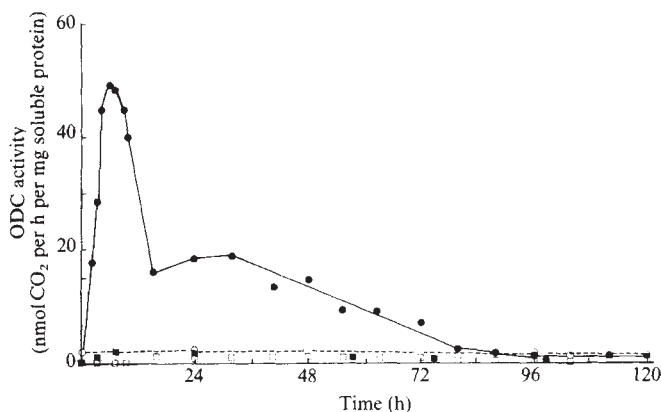


Fig. 1 Specific activity of ornithine decarboxylase in PC12 cultures after various times of treatment as indicated. PC12 cells (~250 generations after their isolation) were cultured on collagen-coated 35-mm tissue culture dishes (Falcon) as described previously^{6,7}, in 1.5 ml of medium. Because the introduction of fresh complete growth medium (85% RPMI 1640, 10% horse serum and 5% fetal calf serum)⁶ to cultures itself caused a 15-fold induction of ODC activity within 6 h, the experiments shown here used cultures maintained in medium containing 1% horse serum. The latter medium did not induce ODC activity and hence long-term experiments (in which medium was changed every other day) could be carried out with a stable baseline. Although growth in such medium was slowed (doubling time ~6 d), neither cell viability nor responsiveness to NGF were affected. (It may also be noted that qualitatively identical results to those shown here were also obtained with cultures maintained in complete growth medium.) Cultures treated with 5-hexyne-1,4-diamine or 1,3-diaminopropane (Aldrich) were preincubated with these compounds for 14 and 1 h, respectively, before addition of NGF. Mouse salivary gland 2.5S NGF¹⁰ was added in 5 μ l of culture medium to a final concentration of 50 ng ml⁻¹. Addition of the same volume of culture medium containing the diluted buffer (0.1 M sodium acetate, pH 5.5) in which the stock NGF (0.5 mg ml⁻¹) was dissolved had no effect on ODC activity. After various times of treatment, cultures were collected by scraping the cells into 400 μ l of assay buffer (see below). The cells were broken by sonication, and the sonicates quick-frozen on dry ice and stored at -20 °C (<7 d) until assayed. Before assay, samples were thawed and centrifuged for 10 min at 12,000 g in a Brinkmann micro-centrifuge. ODC activity was measured in the supernatant fraction by minor modifications of the assay described by Pegg and Williams-Ashman¹¹. Assay buffer was 50 mM Tris-Cl, pH 7.4, containing mM sodium EDTA, 5 mM dithiothreitol, 0.1 mM pyridoxal phosphate and 1 mM D,L-ornithine. For each assay, 300 μ l of cell supernatant (or of buffer for blanks), 150 μ l of assay buffer and 50 μ l of assay buffer containing 0.45 μ Ci of [1-¹⁴C]-D,L-ornithine (specific activity 53 mCi mmol⁻¹; New England Nuclear) were mixed and incubated for 1 h at 37 °C in a disposable 17 $\times$ 100-mm polystyrene tube (Falcon) with a centre well suspended from a rubber stopper (Kontes Glass). Released ¹⁴CO₂ was trapped in 100 μ l of NCS solubilizer (Amersham) absorbed on a piece of filter paper in the centre well. Assays were terminated by injecting 0.5 ml of 50% trichloroacetic acid into the tube through the stopper. After overnight incubation, the trapped ¹⁴CO₂ was counted using Instagel scintillant (New England Nuclear). Assays were linear for the range of protein used (60–300 μ g per sample). Specific activities are based on protein concentrations determined by the method of McGuire *et al.*¹². Values are averages of determinations on duplicate cultures. ○, Untreated; ●, NGF; □, NGF + 1 mM 1,3-diaminopropane; ■, NGF + 0.1 mM 5-hexyne-1,4-diamine.

Figure 1 shows the results of an experiment in which ODC activity was measured in PC12 cultures at various times of treatment with or without 50 ng per ml 2.5S mouse submaxillary gland NGF¹⁰. In NGF-containing cultures, ODC activity underwent a rapid increase which was maximal within 5–8 h of treatment. A smaller secondary increase was also present during the second day of exposure. For 12 separate experiments, the ODC activities ($\pm$ s.e.m.) in cultures treated with or without NGF for 6 h were 41.0 ± 3.1 and 2.2 ± 0.5 nmol CO₂ released per h per mg protein, respectively. This rapid NGF-dependent increase in ODC activity seemed to require RNA transcription, as it was completely blocked (to levels of <0.9 nmol CO₂ released per h per mg protein) by exposure to the RNA synthesis inhibitors actinomycin-D (1 μ g ml⁻¹) or camptothecin (10 μ g ml⁻¹).

The effect of NGF on ODC activity was not generalised to all cell types. Exposure to NGF for 6 h did not alter the ODC activities in cultures of two cell lines (3T3 fibroblasts¹³ or C₆ glioma cells¹⁴) which have no known biological response to the factor. The mean ($n=3$) ODC activity $\pm$ s.e.m. (in nmol CO₂ formed per hr per mg protein) in 3T3 cultures was 20.5 ± 0.7 (no NGF) and 21.0 ± 1.2 (1,000 ng ml⁻¹ NGF) and in C₆ cultures 5.3 ± 0.7 (no NGF) and 4.8 ± 0.2 (1,000 ng ml⁻¹ NGF).

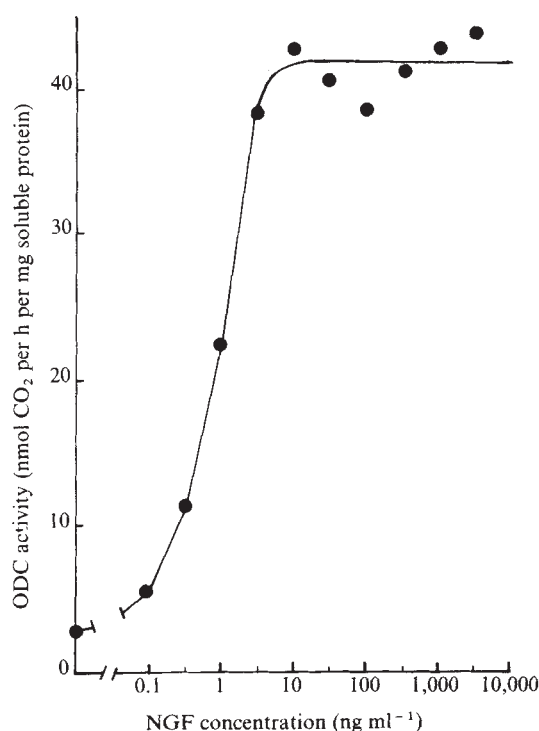


Fig. 2 ODC activity in sister PC12 cultures 6 h after exposure to the indicated concentration of NGF. Cultures were maintained as in Fig. 1 and assays were carried out as described in the legend to Fig. 1. Values are averages of determinations on duplicate cultures.

Figure 2 shows a dose-response relationship (after 6 h of treatment) between NGF concentration and ODC activity in PC12 cultures. Stimulation of activity was detectable at the lowest NGF concentration tested (0.1 ng ml⁻¹) and was maximal at 3–10 ng ml⁻¹ (0.1–0.3 nM) NGF. The latter concentrations correspond to those required for stimulation of neurite outgrowth from PC12 cells^{6,15} and are similar to the dissociation constant reported for NGF with membrane receptors on mammalian sympathetic ganglia¹⁶.

The finding that NGF treatment causes a rapid increase in ODC activity of responsive cells raises the question as to whether such an increase is required for other responses to the factor. To test this, two methods were used to suppress ODC

activity in PC12 cells, treatment with 1 mM 1, 3-diaminopropane (DAP) or with 0.1 mM 5-hexyne-1,4-diamine (HDA). The former suppresses ODC activity by apparently causing a specific inhibition of synthesis of the enzyme^{17,18}, and the latter is a specific, irreversible inhibitor of ODC activity¹⁹ that is effective within living cells (R. Rando, personal communication). As shown in Fig. 1, treatment of PC12 cultures with either compound totally blocked the effect of NGF on ODC activity. At 6 h after addition of NGF, ODC activities in DAP- and HDA-treated cultures were 1 and 4%, respectively, of those in non-inhibited NGF-treated cultures. Nevertheless, treatment with these inhibitors did not affect either cell viability or the ability of NGF to promote neurite outgrowth. Figure 3 illustrates the morphological response of PC12 cells to NGF with or without continuous suppression of ODC activity.

Table 1 Effect of inhibitors of ODC activity on NGF-dependent survival of PC12 cells in serum-free medium

Addition to culture medium (RPMI 1640)	Cells per dish ($\times 10^{-4}$)
None	0.3 ± 0.2
0.1 mM 5-hexyne-1,4-diamine	0.3 ± 0.1
1.0 mM 1,3-diaminopropane	0.2 ± 0.1
50 ng ml ⁻¹ NGF	10.6 ± 1.7
50 ng ml ⁻¹ NGF + 0.1 mM 5-hexyne-1,4-diamine	12.2 ± 2.3
50 ng ml ⁻¹ NGF + 1.0 mM 1,3-diaminopropane	9.4 ± 1.5

PC12 cells (8×10^4) were plated in serum-free culture medium in the presence of the indicated compounds and maintained for 7 d. Cell number per dish was then determined as described elsewhere⁶. Values are means $\pm$ s.e.m. of four determinations.

As may be seen in Fig. 3, NGF treatment also causes an increase in the size of PC12 cell bodies⁸. This increase still occurred when induction of ODC activity was blocked. Measurement of cell body diameters (where diameter is defined as (length of short axis + length of long axis)/2) from photographs of cells gave mean $\pm$ s.e.m. ($n = 25$ in each case) values of $9.4 \pm 0.2 \mu\text{m}$ for untreated cells, and $13.6 \pm 0.5 \mu\text{m}$, $13.9 \pm 0.4 \mu\text{m}$ and $13.5 \pm 0.4 \mu\text{m}$, respectively, for cultures treated for 6 d with NGF, NGF + 1 mM DAP, and NGF + 0.1 mM HDA. Such results did not seem to be due merely to cell flattening, as similar changes in size were also apparent when the cells were detached from the dish and allowed to round up in suspension.

Another effect of NGF on PC12 cells is to promote their survival (as well as their morphological differentiation) in serum-free medium¹⁵. That is, if serum is withdrawn from PC12 cultures, the cells die. However, in the presence of NGF in serum-free medium, the cells survive and extend neurites. In the present experiments, NGF was found to induce ODC in serum-free conditions. The average ODC activities in duplicate cultures treated for 6 h with or without NGF in serum-free culture medium were 26.8 and 1.8 nmol CO₂ released per h per mg protein, respectively. However, as shown in Table 1, suppression of ODC activity in serum-free PC12 cultures by either DAP or HDA did not block the effect of NGF on cell survival.

Experiments were also carried out with dissociated cell cultures of 11-d chick embryo sympathetic neurones. These cells require NGF for their survival and neurite outgrowth^{3,20}. Inclusion of either mM DAP or 0.1 mM HDA in NGF-containing culture medium at the time of plating of such cells did not have an apparent effect on their survival or neurite outgrowth (see Fig. 3) even though ODC activity (measured at 6 d after plating) was suppressed to only 2 and 12%, respectively, of that in control cultures (38.4 pmol CO₂ released per h per mg protein).

Our findings strongly suggest that although NGF can induce ODC activity in responsive cells, neither increased levels of the enzyme molecule nor of its enzymatic activity are required for NGF-dependent survival or stimulation of neurite outgrowth.

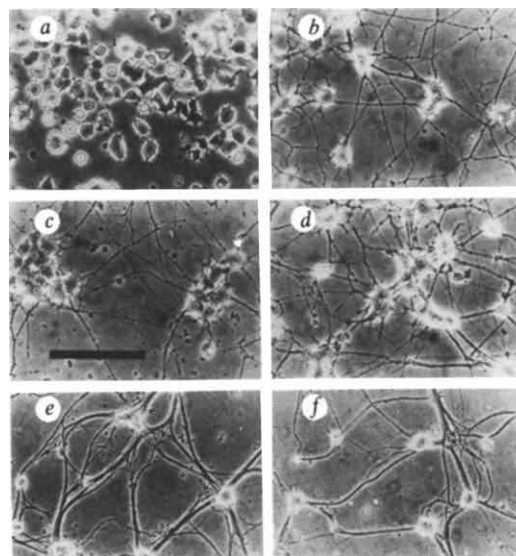


Fig. 3 Phase-contrast micrographs of PC12 cells maintained in culture conditions as described in Fig. 1 and treated for 6 d with (A) no NGF, (B) NGF, (C) NGF plus 1 mM 1,3-diaminopropane, or (D) NGF plus 0.1 mM 5-hexyne-1,4-diamine, and of dissociated cell cultures of 11-d embryonic chick sympathetic neurones maintained for 6 d (E) with NGF or (F) with NGF plus 1 mM 1,3-diaminopropane. Cultures of chick sympathetic neurones were prepared as previously described²⁰ except that the presence of non-neuronal cells was substantially reduced by treatment with 0.6 μM cytosine arabinoside on days 2–4 *in vitro*. Sympathetic neurones treated with 0.1 mM 5-hexyne-1,4-diamine resembled those shown in (E) and (F). Treatment with the ODC inhibitors in the absence of NGF promoted neither neurite outgrowth from PC12 cells nor survival of neurones. Bar, 100 μm .

This conclusion raises several alternatives concerning the role of ODC induction in the mechanism of action of NGF. One is that induction of ODC is causally related to other effects of NGF which were not tested here. Another possibility is that ODC induction bears no causal relationship to other actions of NGF and that it is itself a 'dead end' or terminal consequence of other events related to the interaction of NGF with its target cells. The latter possibility raises the issue of the relevance of ODC induction to the mechanism of action of other agents^{1,2} which affect cell differentiation.

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- Jänne, J., Pösö, H. & Raina, A. *Biochim. biophys. Acta* **473**, 241–293 (1978).
- Russell, D. H., Byus, C. V. & Manen, D.-A. *Life Sci.* **19**, 1297–1306 (1976).
- Levi-Montalcini, R. & Angeletti, P. U. *Physiol. Rev.* **48**, 534–569 (1968).
- MacDonnell, P. C., Nagaiah, K., Lakshmanan, J. & Guroff, G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4681–4684 (1977).
- Lewis, M. E., Lakshmanan, J., Nagaiah, K., MacDonnell, P. C. & Guroff, G. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1021–1023 (1978).
- Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424–2428 (1976).
- Greene, L. A. & Rein, G. *Brain Res.* **129**, 247–263 (1977).

8. Dichter, M. A., Tischler, A. S. & Greene, L. A. *Nature* **268**, 501-504 (1977).
9. Levi-Montalcini, R. & Angeletti, P. U. *Dev. Biol.* **7**, 653-659 (1963).
10. Bocchini, V. & Angeletti, P. U. *Proc. natn. Acad. Sci. U.S.A.* **64**, 787-794 (1969).
11. Pegg, A. E. & Williams-Ashman, H. G. *Biochem. J.* **108**, 533-539 (1968).
12. McGuire, J., Taylor, P. & Greene, L. A. *Analyt. Biochem.* **83**, 75-81 (1977).
13. Todaro, G. J. & Green, H. *J. Cell Biol.* **17**, 299-313 (1963).
14. Benda, P., Lightbody, J., Sato, G., Levine, L. & Sweet, W. *Science* **161**, 370-371 (1968).
15. Greene, L. A. *J. Cell Biol.* **78**, 747-755 (1978).
16. Banerjee, S. P., Snyder, S. H., Cuatrecasas, P. & Greene, L. A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2519-2523 (1973).
17. Póssó, H. & Jänne, J. *Biochem. biophys. Res. Commun.* **69**, 885-892 (1976).
18. Sunkara, P. S., Rao, P. N. & Nishioka, K. *Biochem. biophys. Res. Commun.* **74**, 1125-1133 (1977).
19. Metcalf, B. W. *et al. J. Am. chem. Soc.* **100**, 2551-2553 (1978).
20. Greene, L. A. *Dev. Biol.* **58**, 96-105 (1977).

Cyclic-AMP-induced differentiation in neuroblastoma is independent of cell division rate

CLOINED, cultured mouse neuroblastoma cells, when grown in conditions which restrict cell division, extend neurites¹⁻⁶ and increase the activity of neurotransmitter-related enzymes⁷⁻⁹. Treatments that can induce this differentiation include lowered serum concentrations³, prostaglandins⁴, phosphodiesterase inhibitors⁵ and analogues of cyclic-3',5'-AMP (cyclic-AMP)^{6,10-11}. These treatments have been proposed to act by elevating intracellular cyclic-AMP levels, but because they also inhibit cell division it has been difficult to determine whether their effects are due directly to cyclic-AMP or indirectly to inhibition of cell division¹². As neurite growth and enzyme elevation occur during the interphase portion of the cell cycle (and are retarded during cell division), cyclic-AMP may simply allow differentiation by stopping the cell cycle in interphase³. The butyryl analogues of cyclic-AMP, in particular, markedly restrict cell division (ref. 10; see also Fig. 1a). Because butyrate, which can dissociate from the dibutyryl analogue of cyclic-AMP, can itself markedly inhibit cell division we reasoned that other analogues may exist which are not inhibitors of cell division but which effectively mimic the action of cyclic-AMP. We report here that 8-bromo-cyclic-3',5'-AMP (8Br-cyclic-AMP) effects biochemical and morphological changes in the neuroblastoma clone NBD-2 (ref. 9) without concomitant inhibition of cell division.

As shown in Fig. 1a, 8Br-cyclic-AMP (up to 1 mM) does not inhibit the rate of cell division in this neuroblastoma culture but, in fact, below 0.3 mM produces an increase in the cell division rate (150-160% of control). In contrast, both dibutyryl-cyclic-AMP (Bt₂-cyclic-AMP) and Na butyrate inhibit cell division. At 1 mM, Bt₂-cyclic-AMP inhibits division ~50% and Na butyrate inhibits cell division almost completely. (0.2 mM Na butyrate produces an inhibition of cell division comparable to 1 mM Bt₂-cyclic-AMP.)

Table 1 Enzyme activities in the NBD-2 cells

Treatment	TH Product per h per 10 ⁶ cells† (pmol)	CAT (pmol)	AChE (μmol)	Cell no. per culture*
Control	50 ± 9	3.3 ± 0.3	5 ± 3	6.4 ± 0.8
1 mM 8Br-cyclic-AMP	810 ± 12	9.9 ± 1.0	6 ± 3	6.2 ± 1.1
1 mM Bt ₂ -cyclic-AMP	760 ± 10	10.2 ± 0.5	120 ± 11	2.9 ± 0.3
0.2 mM butyrate	62 ± 8	3.9 ± 0.8	6 ± 4	2.9 ± 0.5

NBD-2 cells were plated at 0.4×10^6 cells per 60 mm diameter culture dish in 5 ml of F-12 medium supplemented with 10% newborn calf serum. Treatments were added after 24 h, and cells were collected for assay 48 h later. The enzyme assays used are described in detail elsewhere⁹.

* Values are expressed in terms of 10^6 cells after 72 h in culture per 10^6 cells originally plated.

† All values represent the mean ± SEM of four determinations.

On the other hand, 8Br-cyclic-AMP and Bt₂-cyclic-AMP, but not Na butyrate, induce comparable increases in neurite extension (Fig. 1b). Thus, the cyclic-AMP moiety, as opposed to decreased cell division, seems to be responsible for the morphological changes in these cells. However, because the data in Fig. 1 are taken from a population of cells at a single time point, it is still possible that only some of the cells undergo neurite extension (and diminished cell division) while other cells increase their rate of division. For example, at low 8Br-cyclic-AMP concentrations (little effect on neurite extension) perhaps a small, more rapidly dividing subpopulation is being revealed, and as the 8Br-cyclic-AMP concentration is raised this effect becomes relatively balanced by another subpopulation of cells now undergoing neurite extension (and not dividing).

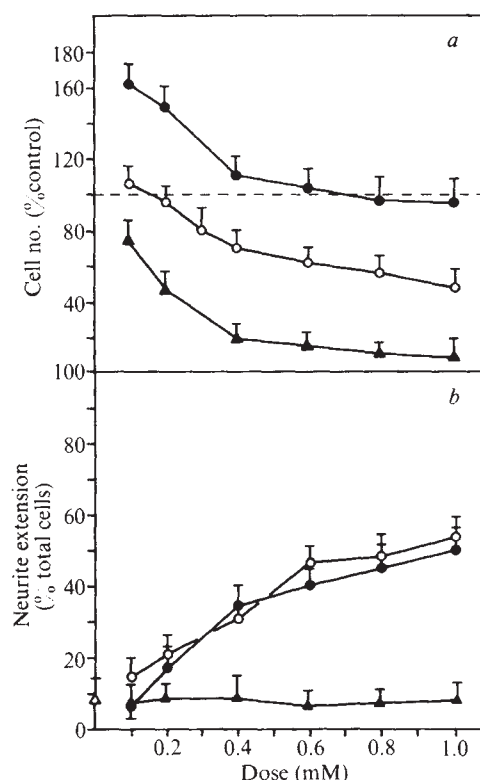


Fig. 1 Cell division and neurite extension in cultured NBD-2 neuroblastoma cells. NBD-2 neuroblastoma cells, isolated as previously described⁹, were plated at 0.4×10^6 cells per 60-mm diameter culture dish in 5 ml of F-12 medium supplemented with 10% (v/v) GG-free newborn calf serum. Treatments were begun 24 h later and continued for 48 h. Drugs and media were changed every 24 h. *a*, Cell numbers were determined by haemocytometry on the basis of trypan blue exclusion⁹. More than 99% of the cells were trypan blue excluding. Values represent the mean ± SEM of four separate experiments in which duplicates were run for each determination. *b*, The number of cells extending neurites was determined in each sample by counting the number of cells (out of 200) with processes greater than twice the length of the cell body. Values represent the mean ± SEM of four separate experiments as in *a*. Δ, Control; ○, Bt₂-cyclic-AMP; ●, 8Br-cyclic-AMP; ▲, butyrate.

Examination of 8Br-cyclic-AMP-treated neuroblastoma cells with time-lapse cinematography (3 d) revealed, however, that every cell within the microscopic field underwent both neurite extension and division during each 24-h period. Figure 2 is taken from one time-lapse film and shows that the 8Br-cyclic-AMP-treated cells exhibit both neurite extension and cell division. The cells spent the majority of time in a differentiated state (Fig. 2a,d,j) and, with rare exception, were de-differentiated only immediately before and after division. For example, the average

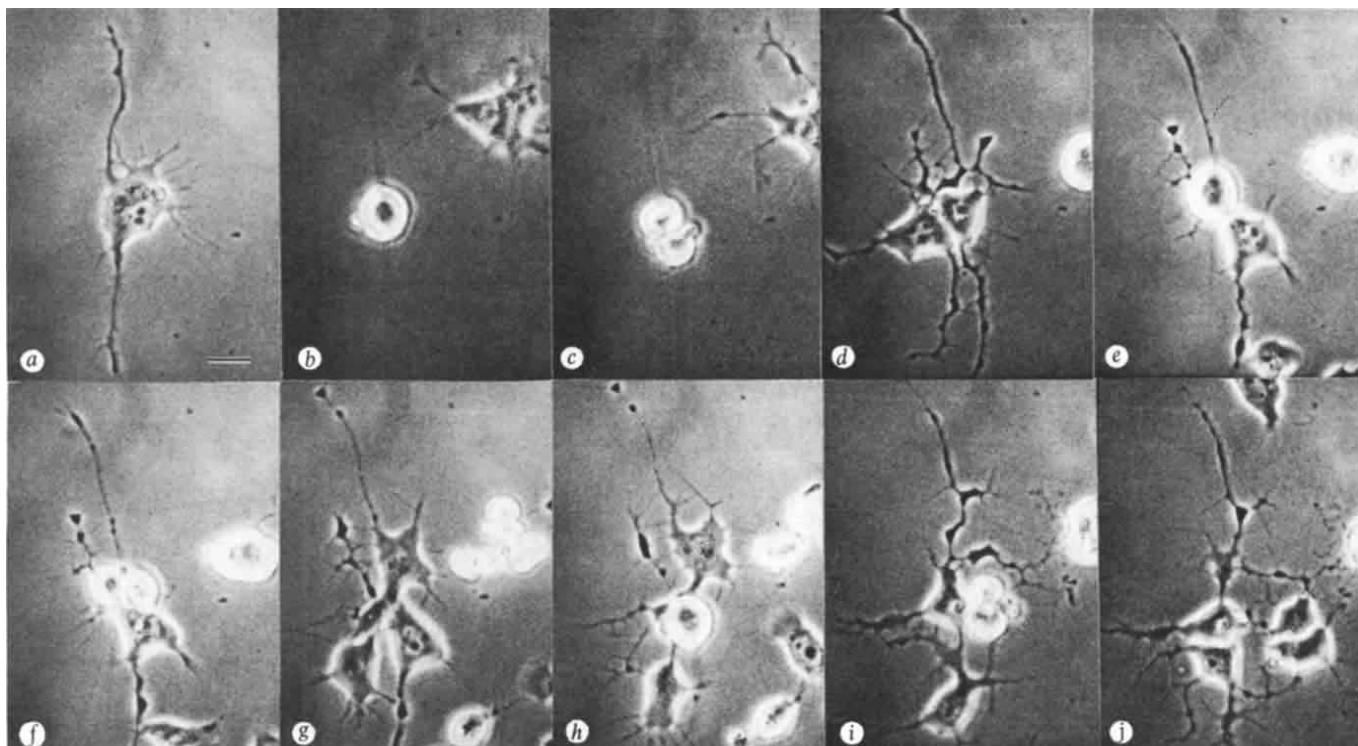


Fig. 2 Differentiation and division in NBD-2 cells treated with 8Br-cyclic-AMP. NBD-2 cells were plated at 0.1×10^6 cells per 2 ml F-12 medium supplemented with 10% newborn calf serum in Rose chambers and gassed with 5% CO_2 95% air. After 24 h, the cells were treated with 1 mM 8Br-cyclic-AMP and time-lapse cinematography was begun ~ 10 h later. For the first 15 h (a) the cell remained in a differentiated state. It withdrew its processes and remained rounded up (b) for 40 min whereupon it divided (c). After 50 min both cells exhibited processes at least twice the diameter of the cell body (d). The cells remained in this differentiated state for 18 h until one of the cells de-differentiated and divided over a 54-min period (e, f). The newly divided cells differentiated (g, 100 min) and the second cell subsequently de-differentiated (h, 2 h) and divided (i, 40 min). The cells differentiated (j) and all four cells remained differentiated for the duration of this particular film (>15 h). The photography was taken on a Zeiss RA microscope with phase contrast optics in a hot room at 37°C . A Sage series 500 cinephotomicrographic apparatus with a Bolex H167 16-mm movie camera was used with 0.5 s exposures at 1/s. The calibration bar represents $10\text{ }\mu\text{m}$.

time in a de-differentiated state was 77 ± 12 min ($n=10$ randomly selected divisions).

Stopped-frame analysis at 4-h intervals showed that 40–60% of the treated cells exhibited neurite extension at any given time, in agreement with Fig. 1b. Thus, as this division rate is comparable to untreated cells⁹ and as growth curves of 8Br-cyclic-AMP-treated and untreated cells are identical (data not shown), the increase in neurite extension cannot be attributed to a decrease in division rate *per se*.

Concomitant with the changes in cell morphology produced by the two cyclic-AMP analogues, tyrosine hydroxylase (TH, EC 1.14.16.2) and, to a lesser extent, choline acetyltransferase (CAT, EC 2.3.1.6) activities are increased (Table 1). The fact that these changes in enzyme levels are induced by both 8Br-cyclic-AMP and Bt_2 -cyclic-AMP (but not Na butyrate at a concentration that produces an inhibition of cell division equivalent to 1 mM Bt_2 -cyclic-AMP) implies that the cyclic-AMP moiety of these compounds is also capable of modifying the biochemistry of the cultured neuroblastoma cells in a manner independent of an effect on cell division rate. Interestingly, this was not the case for a third enzyme examined. 1 mM Bt_2 -cyclic-AMP, but not 0.2 mM Na butyrate or 1 mM 8Br-cyclic-AMP, dramatically elevated the activity of acetylcholinesterase (AChE, EC 3.1.1.7) (Table 1). Although the elevation of this enzyme is often associated with decreased cell division^{7,13–15}, the lack of effect of Na butyrate (0.2 mM, matched for effects on cell division) argues against a simple cause. However, as higher Na butyrate concentrations do induce comparable increases in AChE activity (not shown), some butyrate (either attached or dissociated from cyclic-AMP) or butyrate-cyclic-AMP interaction may be responsible for this effect.

These results, then, strongly support the contention that cyclic-AMP can influence neuroblastoma morphology and biochemistry in a manner independent of changes in cell division rate. Further, 8Br-cyclic-AMP seems to be better suited than at least the dibutyl analogue of cyclic-AMP for studies in which effects on cell division may complicate interpretation of the results.

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1. Prasad, K. N. *Nature* **234**, 471–473 (1971).
2. Byfield, J. E. & Karlsson, U. *Cell Differentiation* **2**, 55–64 (1973).
3. Seeds, N. W., Gilman, A. G., Amano, T. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **66**, 160–167 (1970).
4. Prasad, K. N. *Nature new Biol.* **236**, 49–52 (1972).
5. Furmanski, P., Silverman, D. J. & Lubin, M. *Nature* **233**, 413–415 (1971).
6. Prasad, K. N. & Hsie, A. W. *Nature new Biol.* **233**, 141–142 (1971).
7. Blume, A. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **67**, 786–792 (1970).
8. Sheppard, J. R. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1316–1320 (1971).
9. Waymire, J. C. & Gilmer-Waymire, K. N. *J. Neurochem.* **31**, 693–698 (1978).
10. Waymire, J. C., Gilmer-Waymire, K. N. & Boehme, R. *J. Neurochem.* **31**, 699–705 (1978).
11. Waymire, J. C. *et al. Molec. Pharmac.* (in the press).
12. Miller, R. A. & Ruddle, F. H. *J. Cell. Biol.* **63**, 295–299 (1974).
13. Schubert, D., Tarikas, H., Harris, A. J. & Heinemann, S. *Nature new Biol.* **233**, 79–80 (1971).
14. Amano, T., Richelson, E. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 258–263 (1972).
15. Harkins, J., Arsenault, M., Schlessinger, K. & Kates, J. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3161–3164 (1972).

Free ubiquitin is a non-histone protein of trout testis chromatin

A NON-HISTONE chromosomal protein extracted from trout testis chromatin by 0.35 M salt, protein S, (ref. 1) has now been purified to homogeneity and its primary structure determined (Fig. 3) and shown to be identical with that of the thymus protein, ubiquitin²⁻⁴ (originally called thymopoiitin) and sequenced by Schlesinger *et al.*⁵ Ubiquitin was previously reported in chromatin by Goldknopf *et al.*⁶ as a component of protein A-24 in which it is covalently bound by the α -carboxyl group of its C-terminal glycine to the ϵ -amino group of lysine 119 of histone H2A in an isopeptide linkage⁷. Goldknopf and Busch⁸ have reported that protein A-24 is a component of the core nucleosomes of rat liver chromatin being present in quantities sufficient for it to be linked to H2A in 10–20% of the total nucleosomes. The present studies indicate clearly that ubiquitin can also occur as a free polypeptide in trout testis chromatin and is readily extracted by 0.35 M NaCl. Moreover, free trout testis ubiquitin (protein S) is rapidly released together with HMG-T (ref. 9) (and small amounts of H1) during limited micrococcal nuclease digestion of trout testis chromatin and is probably also located in the linker regions of the chromatin. DNase I digestion does not release free ubiquitin preferentially¹⁰. In view of the pronounced biological effects of free ubiquitin on membrane adenylyl cyclase of immature thymocytes^{3,4} and the ability of ubiquitin to promote the differentiation of these cells into mature T cells^{3,4}, its occurrence in chromatin raises the possibility of a role of ubiquitin in cell differentiation and differential gene expression.

Trout testis ubiquitin (protein S) was isolated from washed chromatin by the method described by Goodwin *et al.*¹¹ for preparation of calf thymus HMG-proteins and was purified by chromatography on a BioRex 70 cation exchanger¹ (Fig. 1); ubiquitin was eluted as a sharp peak at the passthrough volume of the column and on electrophoresis either in starch¹² (Fig. 2a), Triton-polyacrylamide¹³ gels (Fig. 2b) or SDS-polyacrylamide gels¹⁴ (Fig. 2c), it migrated as a single component with a minor slower running contaminant. The mobility of trout testis ubiquitin is identical with that of authentic calf thymus ubiquitin (Fig. 2b). Comparison with the mobility of the molecular weight

Fig. 1 Ion-exchange chromatography of trout testis HMG-proteins on a column (2 × 50 cm) of BioRex 70 (200–400 mesh) eluted with a linear gradient formed by mixing 500 ml of 2% with 500 ml of 11% guanidinium chloride¹. The fractions containing the protein peak emerging at the pass-through volume of the column (S) were combined, exhaustively dialysed in membranes of Sephadex III (MW cutoff 3,500) against distilled water and lyophilised. The yield of protein S was approximately 17 mg per kg trout testis.

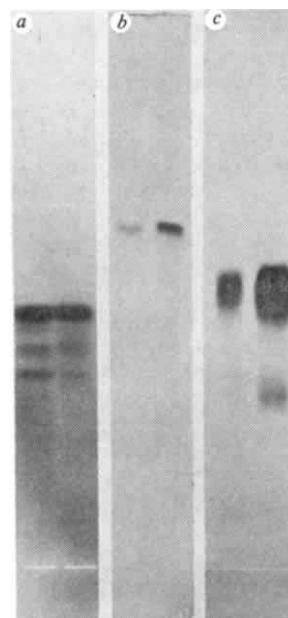
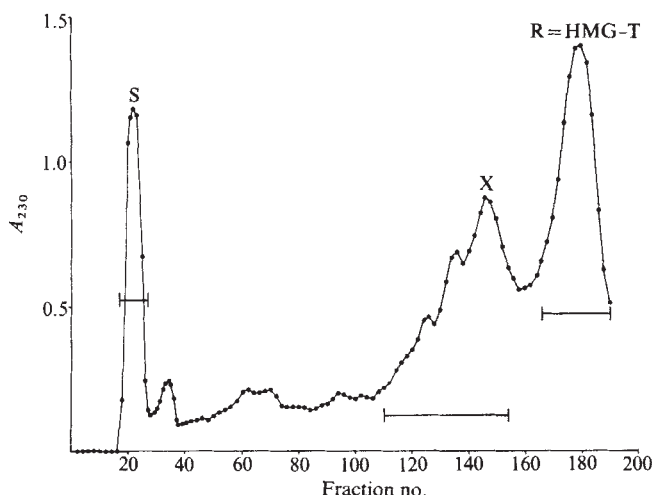


Fig. 2 Gel electrophoresis in three systems of trout testis ubiquitin (protein S) with markers of authentic calf thymus ubiquitin. *a*, Starch gel electrophoresis in aluminium lactate buffer¹². *b*, Triton-polyacrylamide¹³ gel electrophoresis (15% polyacrylamide, 0.22% Triton X-100, 5% acetic acid). *c*, SDS-polyacrylamide¹⁴ gel electrophoresis (15% polyacrylamide, 1% SDS, Tris-glycine, pH 6.8). Molecular weight markers of insulin (6,000), lysozyme (14,300) and cytochrome *c* (12,000) were included in order to estimate the MW of the ubiquitins. The ubiquitin bands in (*c*) were excised, hydrolysed¹⁵ and their amino acid compositions determined using a Beckman Model 121 M analyser equipped with a Systems AA integrator. Amino acid compositions were reduced to the most likely stoichiometry using the least squares fit program of Osawa and Tanaka²⁴.

protein markers on the SDS-polyacrylamide gel (Fig. 2c) gives molecular weights of 8,500 for both trout testis and calf thymus ubiquitin.

Amino acid analyses show that trout testis and calf thymus ubiquitin are identical in composition within experimental error. In these analyses, the trout testis ubiquitin major component was eluted from the SDS-polyacrylamide gel and hydrolysed¹⁵. We have also carried out a completely independent amino acid sequence analysis of trout testis ubiquitin by automatic Edman degradation of the intact protein as well as a series of peptides obtained by trypsin cleavage after protection of the lysyl residues by maleylation⁵, by chymotryptic cleavage, by cleavage with staphylococcal protease¹⁶, by chemical cleavage at aspartyl residues in 0.2 M acetic acid¹⁷ and by chemical cleavage at the single tyrosyl residue at position 59 with *N*-bromosuccinimide. The single methionyl residue is amino-terminal and it was found that cleavage with cyanogen bromide yielded free homoserine lactone and a fragment (residues 2–74) indistinguishable from the intact protein by gel electrophoresis.

The only area of uncertainty in the sequence is between residues 68 and 71, as it has not yet been possible to recover from trout testis ubiquitin a peptide containing the histidyl residue located at position 68 of calf thymus ubiquitin. A summary of the peptides leading to the deduction of the complete sequence of trout testis ubiquitin is given in Fig. 3. Details of the sequence analysis will be published elsewhere. This sequence is identical to that described for calf thymus ubiquitin by Schlesinger *et al.*⁵ with the possible exception of residues 68–71. Our independent determination of the complete sequence of ubiquitin from a salmonid fish testis and the demonstration of the identity of at least 71 of the 74 positions with ubiquitin from calf thymus shows that the structure of ubiquitin is probably as highly conserved during evolution as that of the arginine-rich histones H3 and H4. A limited

N-terminal sequence is available for a plant ubiquitin (from celery)⁴ and shows that six of the eight residues in this region are identical but the sequence is too limited to make a conclusion about the evolutionary conservation of ubiquitin in the plant kingdom. Note that a radioimmunoassay developed by Goldstein *et al.*⁴ for ubiquitin detects the protein in vertebrates, invertebrates, plants, fungi and a variety of bacteria so it is likely that there has been only limited divergence in the major antigenic determinants during evolution.

Ubiquitin has been noted previously as a constituent of protein A-24 of calf thymus or rat liver nuclei^{6,8} in which it is covalently bound by an iso-peptide linkage⁷ from a C-terminal glycine residue to the ϵ -amino group of lysine 119 of histone H2A to produce a branched protein. The identification of one 'arm' of this protein with calf thymus ubiquitin was made when Hunt and Dayhoff¹⁸ noted that a sequence of 40 residues at the N-terminus of protein A-24 was identical with calf thymus ubiquitin (the H2A arm is blocked to Edman degradation in the sequencer by its N-terminal acetyl group). The molecular weight of A-24 has been reported as 27,000 by SDS-polyacrylamide electrophoresis⁶ but there is a discrepancy between this value and the sum of the molecular weights of ubiquitin (8,451) and H2A (14,000). It is possible that A-24 may migrate anomalously in SDS-polyacrylamide gels, as both components, ubiquitin and H2A have strongly hydrophobic regions and could bind additional detergent molecules. The second discrepancy is that Schlesinger *et al.*⁵ originally reported that the C-terminal residue of calf thymus ubiquitin was arginine, whereas the peptide containing the isopeptide linkage isolated by Goldknopf and Busch⁷ was reported as comprising a sequence Gly-Gly, presumably at the C-terminus of the ubiquitin arm. In our sequence studies of trout testis ubiquitin we have noted heterogeneity at the C-terminus, 50% of molecules are terminated by arginine (Fig. 3, peptide CH-IV-1) and 50% by glycine (Fig. 3,

Table 1 Amino acid composition of calf thymus ubiquitin and trout testis protein S

	Calf thymus ubiquitin		Trout testis protein S	
	Moles %	Residues	Moles %	Residues
Asp	9.81	(7)	9.05	(7)
Thr	9.31	(7)	8.77	(7)
Ser	4.23	(3)	4.34	(3)
Glu	15.57	(12)	15.97	(12)
Pro	3.72	(3)	3.97	(3)
Gly	6.77	(5)	6.56	(5)
Ala	2.71	(2)	2.59	(2)
Val	5.41	(4)	5.08	(4)
Met	0.85	(1)	1.20	(1)
Ile	8.80	(7)	9.79	(7)
Leu	11.68	(9)	12.56	(9)
Tyr	1.18	(1)	1.39	(1)
Phe	2.54	(2)	2.59	(2)
His	1.35	(1)	1.29	(1)
Lys	9.64	(7)	9.79	(7)
Arg	5.25	(4)	5.08	(4)
	Total	75	Total	75

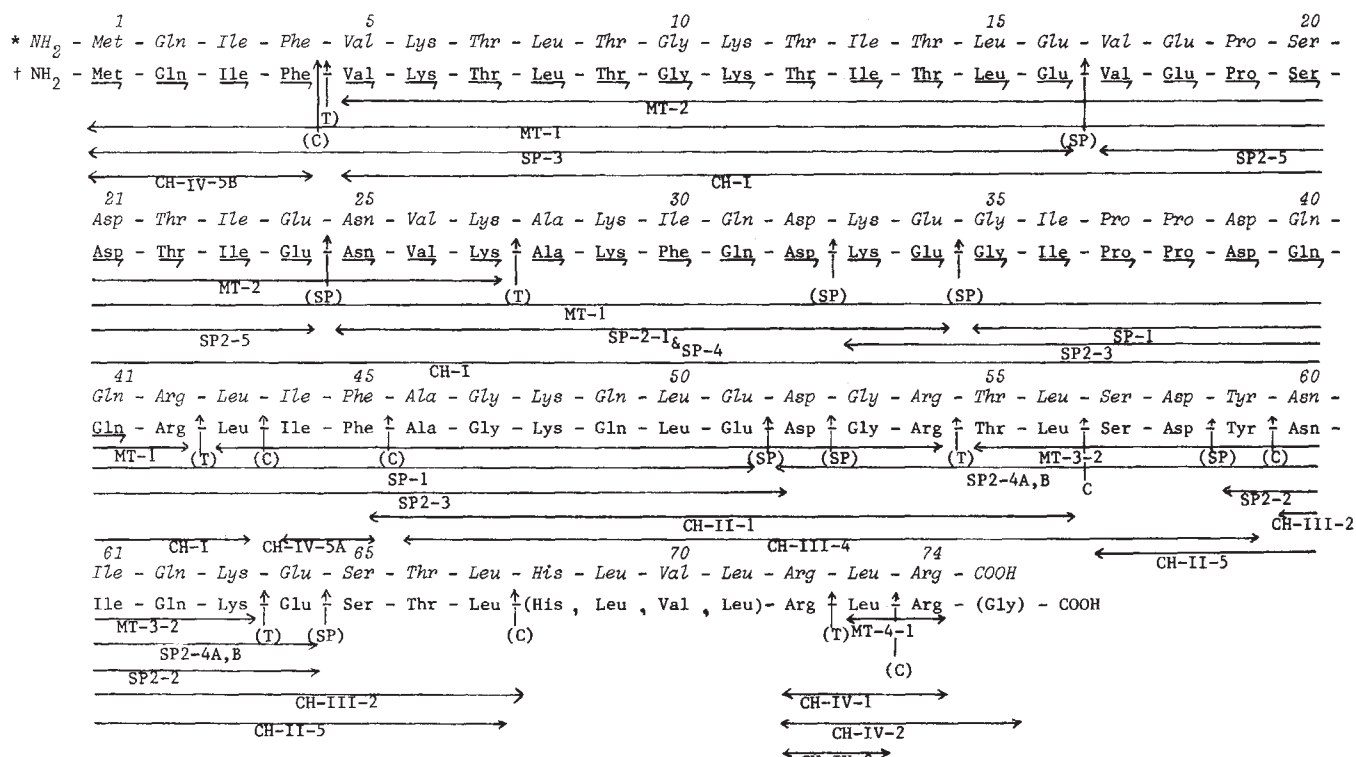
peptide CH-IV-2) but we have not seen evidence of a Gly-Gly sequence at the C-terminus. Further work is required to establish whether protein A-24 contains sequences additional to the reported sequence of calf thymus and trout testis ubiquitin and which might account for the apparent extra size of calf thymus A-24. Application of the method of Goldknopf *et al.*⁶ to trout testis chromatin in our hands has not, so far, yielded a protein corresponding to A-24.

When trout testis nuclei were digested to a limited extent with micrococcal nuclease in conditions which lead to the rapid digestion of the 'linker' regions of chromatin (that is, between nucleosomes)¹⁹⁻²³, we observed that ubiquitin was released into

Fig. 3 Alignment of peptides from trout testis ubiquitin and comparison with the amino acid sequence of calf thymus ubiquitin⁵. MT, peptides from tryptic digestion of maleylated ubiquitin; CH, chymotryptic peptides; SP, staphylococcal protease peptides. Automated sequence determination was performed on purified peptides or intact ubiquitin on a Beckman 890C protein sequencer using a programme similar to that of Edman and Begg²³ but replacing the Quadrol buffer with the volatile DMAA buffer. The 2-anilino-5-thiazolinone derivatives of the amino acids from the sequencer were identified and quantitated by amino acid analysis following back hydrolysis in 47% hydriodic acid at 150° for 20 h.

† Trout 'S'

* Ubiquitin



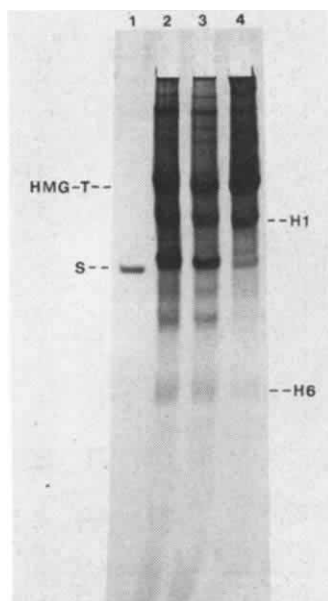


Fig. 4 Electrophoretic analysis of acid soluble proteins solubilised by micrococcal nuclease from trout testis nuclei. Micrococcal nuclease digestion of trout testis nuclei: trout testis nuclei were prepared as described previously¹⁰. The nuclear pellet was washed twice with RSB buffer (0.01 M Tris-HCl, pH 7.4, 0.01 M NaCl, 3 mM MgCl₂). Microscopic examination of the nuclear suspension revealed nuclei free of cytoplasmic tabs. Nuclei were resuspended in RSB buffer containing 1 mM CaCl₂ at a concentration of 10 mg ml⁻¹ (200 A₂₆₀) of DNA and incubated with micrococcal nuclease (Worthington, 250 units ml⁻¹) at 37°C and digested to the extent of 5–10% of the input DNA. The reaction was terminated by chilling the tubes on ice and immediate centrifugation for 5 min at 1,000 g in a Sorvall HB-4 rotor. The supernatant was designated S₁ and used together with the pellet for protein extraction. Acid extraction of proteins and gel electrophoresis: chromosomal proteins were extracted from S₁ and pellet fractions by incubation of that fraction with 0.2 M H₂SO₄ for 1 h at 4°C, as described previously¹⁰. Three batches of trout testis nuclei, collected at different stages of maturation were digested with micrococcal nuclease to the extent of 10–15% solubilisation of the input DNA, as previously described. The acid soluble proteins from the soluble supernatant were analysed in 12% polyacrylamide gels containing 0.22% Triton X-100. This gel system allows complete separation of the histones¹³ and also of HMG-T, ubiquitin (S) and H6. Electrophoresis was for 21 h at 300 V at 4°C. The gel was stained with amido black for 15 h and destained with 5% acetic acid. Slot 1 contains a sample of purified trout testis ubiquitin. Slot 2 shows the proteins solubilised after 15% digestion of middle-late testis nuclei. Slot 3: proteins solubilised after 12% digestion of middle-late testis nuclei. Slot 4: proteins solubilised after 8% digestion of late testis nuclei.

the soluble fraction together with the bulk of the HMG-T. The electrophoretic pattern in a Triton-containing polyacrylamide gel of proteins solubilised by micrococcal nuclease is illustrated in Fig. 4. Slots 2, 3 and 4 contain proteins released by the nuclease from three different batches of nuclei prepared from trout testis at various stages of maturation. In all three cases, it is clear that ubiquitin or protein S is released by the nuclease. The identity of the ubiquitin band was confirmed by excision of the stained bands and then subsequent acid hydrolysis and amino acid composition analysis. Each of the bands in slots 2, 3 and 4 showed amino acid compositions identical with the marker ubiquitin band (slot 1). Examination by gel electrophoresis of the proteins remaining in the nuclear pellet following micrococcal digestion (data not shown) indicates that no detectable band of ubiquitin remains following the nuclease treatment suggesting that ubiquitin is preferentially associated with linker regions in chromatin. However, the fraction represented by ubiquitin of the total proteins released by the nuclease in the soluble fraction varies in different batches of nuclei. This finding is consistent with our previous observations that the major protein released

by micrococcal nuclease, namely HMG-T, also changes in concentration as a function of testis development.

Our finding of a free polypeptide in the low salt extractable non-histone fraction of trout testis chromatin which is virtually identical in primary structure with calf thymus ubiquitin indicates that ubiquitin is a true chromosomal protein which is widely distributed. The extreme conservation of its structure during evolution suggests that like the arginine-rich histones H3 and H4, ubiquitin has a vital structural role in chromatin. The fact that it constitutes only 2–5% of the concentration of the individual nucleosomal core histones further suggests that ubiquitin can only be present in a small sub-set of the chromatin and, therefore, that the conserved structure in which it is located is different from the bulk of the chromatin in the now well-established nucleosomal core and linker structure (beads-on-a-string).

Our experiments with micrococcal nuclease suggest that ubiquitin is present in an easily digested domain of chromatin which contains as a major protein constituent, the non-histone protein HMG-T. Furthermore, we have shown recently that during limited micrococcal digestion of trout testis chromatin, linker regions containing HMG-T (and ubiquitin) are rapidly solubilised and concomitantly, a sub-set of nucleosome core particles containing another non-histone protein, H6, is also released^{22,23}. The DNA sequences present in this sub-set of nucleosomes are enriched five to tenfold in sequences hybridising with testis RNA and this degree of enrichment of DNA sequences is also confirmed by DNA-driven hybridisations^{22,23}. Thus, there seems to be clear evidence of the localisation of three non-histone proteins, ubiquitin, HMG-T and H6 in a domain of chromatin enriched in DNA sequences which can be transcribed into cellular RNA. It is possible that these proteins, either individually or as elements of a pattern could provide recognition signals which are necessary but not sufficient in themselves for transcription of designated regions of chromatin.

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- Marushige, K. & Dixon, G. H. *J. biol. Chem.* **246**, 5799–5805 (1971).
- Goldstein, G. *Nature* **147**, 11–14 (1974).
- Basch, R. S. & Goldstein, G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1474–1478 (1974).
- Goldstein, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 11–15 (1975).
- Schlesinger, D. H., Goldstein, G. & Niall, H. D. *Biochemistry* **14**, 2214–2218 (1975).
- Goldknopf, I. *et al. J. biol. Chem.* **250**, 7128–7187 (1975).
- Goldknopf, I. & Busch, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 864–868 (1977).
- Goldknopf, I., French, M. F., Musso, R. & Busch, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5492–5495 (1977).
- Watson, D. C., Peters, E. H. & Dixon, G. H. *Eur. J. Biochem.* **74**, 53–60 (1977).
- Levy, W. B., Wong, N. C. W. & Dixon, G. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2810–2814 (1977).
- Goodwin, G. H., Nicolas, R. H. & Johns, E. W. *Biochim. biophys. Acta* **405**, 280–291 (1975).
- Sung, M. T. & Smithies, O. *Biopolymers* **7**, 39–58 (1969).
- Rodriguez-Alfageme, C., Zweidler, A., Mahowald, A. & Cohen, L. H. *J. biol. Chem.* **249**, 3729–3736 (1974).
- Shapiro, A. L., Vinuela, E. & Maizel, J. V. *Biochem. biophys. Res. Commun.* **28**, 815–820 (1967).
- Houston, L. L. *Analyt. Biochem.* **44**, 81–88 (1971).
- Houmard, J. & Drapeau, G. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3506–3509 (1972).
- Schroeder, W. A., Shelton, J. R., Shelton, J. B., Cormick, J. & Jones, R. T. *Biochemistry* **2**, 992–1008 (1963).
- Hunt, L. H. & Dayhoff, M. *Biochem. biophys. Res. Commun.* **74**, 650–655 (1977).
- Levy, W. B. & Dixon, G. H. *Nucleic Acids Res.* **4**, 883–898 (1977).
- Levy, W. B., Wong, N. C. W., Watson, D. C., Peters, E. H. & Dixon, G. H. *Cold Spring Harb. Symp. quant. Biol.* **42**, 793–801 (1978).
- Levy, W. B. & Dixon, G. H. *Can. J. Biochem.* **56**, 480–491 (1978).
- Levy, W. B. & Dixon, G. H. *Fedn Proc.* **37**, 1787 (1978).
- Levy, W. B., Connor, W. B. & Dixon, G. H. *J. biol. Chem.* (in the press).
- Ozawa, K. & Tanaka, S. *Analyt. Biochem.* **14**, 270–280 (1968).
- Edman, P. & Begg, G. *Eur. J. Biochem.* **1**, 80–91 (1967).